A STUDY OF THE RELATIONSHIP BETWEEN ABSORPTION OF CALCIUM AND MAGNESIUM IONS AND SAP FLUX IN EXCISED ROOT SYSTEMS OF *ACACIA CYCLOPS*

P. Kuyler

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CONTENTS

ABSTRACT

Single excised root systems of *Acacia cyclops* from plants adapted to different environmental conditions were fed high and low concentrations of calcium and magnesium in four combinations at constant transpiration pressure.

Sap flux and sap calcium and magnesium concentrations were measured and ranked from high to low against each other using associated feeding solutions. A comparison of feeding solutions associated with high or low sap calcium or magnesium did not show significant antagonism. High calcium + high magnesium and low calcium + low magnesium feeding solutions are constantly associated with high and low sap calcium and magnesium concentrations respectively. The relationship between sap flux and sap calcium concentrations for fresh plant root systems indicate that high calcium feeding appears to inhibit sap flux in small plants. High magnesium and calcium feeding solutions were more important for high sap flux in larger fresh plants.

1. INTRODUCTION

1.1 The nature of the problem investigated and the basic approach

The question of a relationship between uptake of salts and uptake of water by plants has been discussed since the dawn of plant physiology. Early workers were somewhat divided as to this relationship. De Saussure (1804) (Bowling 1976) reported a proportional difference in salt and water uptake, while others such as Sacks and Pfeffer thought that a more direct relationship existed.

More recent workers, such as Pitman (1965 and 1966) have recorded that transpiration appears to affect the selectivity of ion absorption. A change in water flux was noted to change the reactive amounts of sodium and potassium being transported into the roots of barley and mustard plants.

In this investigation an attempt to throw some more light on the relationship between water flux and ion uptake will however take an entirely different approach to that which is commonly indicated in the literature. Rather than vary the transpiration rate, the characteristics of the feeding solution will be varied. The specific feeding solution applied may then be used as a common parameter which may be related to measurements of sap flux and sap ion concentration. This may indicate how important the characteristics of the feeding solution are in determining the transport rate of sap as well as the selectivity of ion absorption.

For this investigation excised root systems of *Acacia cyclops* were used. *Acacia cyclops* is a common alien on the Cape Flats, being introduced from Australia during the beginning of the last century (Roux 1961). The prime aim was to stabilize the sand dunes in this region. *Acacia cyclops* was chosen for this study because McKenzie (1975) had already conducted some preliminary studies on cation absorption by single excised roots in this plant.

The biggest difficulty in using excised root systems in a study of water flux and ion uptake is firstly that laboratory experimental conditions must be clearly defined. The problem of physiological stress is also very important and results obtained may in fact be those of a stress reaction, rather than a reflection of the possible situation in the field. In excised root studies the fact that the nutrient supply from the leaves has been cut off must also be accounted for in the results.

1.2 Theoretical background

A review of the literature indicates that at least one membrane must be crossed in the movement of water or ions from the external root environment to the vascular system. The apoplasmic pathway is effectively blocked at the endodermis by suberization of the radial walls of cells (Bidwell 1974, Bowling 1976). This means that the characteristics of this membrane are of fundamental importance in determining how water and ions move into the xylem.

Investigations by Brouwer (1954)(Bowling, 1976) have indicated that water uptake is unaffected by a metabolic inhibitor such as 2,4-dinitrophenol. In these studies on Vicia faba he noticed that chloride uptake was however severely inhibited. This indicates that water uptake may be largely non-metabolic as opposed to ion uptake which is metabolically driven.

The general opinion in the literature appears to be that water flux consists of at least three components. The first exists by virtue of an osmotic pressure difference between the external solution and the xylem fluid (Anderson, 1976). In addition to this, there is a relatively small active component as well as the effect of transpiration, which exists by virtue of the fact that a water column has the ability to transmit tension along it (due to adhesive properties of water molecules). For the purposes of this investigation the transpiration force will be kept constant. The contribution to water flux by the other two components will thus be important.

Workers such as Epstein (1972) have concluded that plant membranes are relatively impermeable when it comes to the transport of inorganic ions across them. The fact that active processes are thought to be important has already been pointed out. The concept of carrier transport has been proposed to account for the high degree of selective ion uptake often accounted for in plants (Epstein, 1972 and Nielsen, 1972). Carrier theory postulates competition by individual ions for carriers or specific carrier sites. This selectivity is thus a product of competition.

In this investigation sap flux will be related to competition by calcium and magnesium at the root membrane, as related to their specific concentrations in the feeding solution.

The interaction between calcium and magnesium has been widely accounted for in the literature. In previous investigations on the uptake of these cations by excised roots of <u>Acacia cyclops</u>, McKenzie (1974) reports that high Ca^{2+} seems to enhance the uptake of Mg^{2+}. Moore et al (1961), on the other hand, report that a large fraction of the magnesium uptake is effectively blocked by small amounts of Ca^{2+} (in studies using excised barley roots). This inhibition of Mg^{2+} uptake by low Ca^{2+} has also been reported for soybeans (Leggett and Gilbert, 1969) and groundnuts (Fageria, 1974). From these observations it is clear that Ca has the ability to modify membrane selectivity with increasing concentration (as was postulated by Jacobson et al (1961).

Regarding the uptake of magnesium and its influence on calcium transport, McKenzie (1974) explains that Mg^{2+} uptake showed a large amount of fluctuation with high and low Ca^{2+} and Mg^{2+} feeding solutions. Mg^{2+} does not appear to inhibit or promote Ca^{2+} uptake in this investigation.

2. METHODS

2.1 Collection of plants

Young 0,3-0,6 m tall Acacia cyclops plants were collected from a site on the Ou Kaapse Weg approximately 1 km from Noordhoek in the Cape Peninsula. They were growing on very sandy soil on embankments next to the road.

Plants were removed by digging up a (0,5m x 0,5m) x 1,0-1,5m deep block of soil around the stem base. This ensured successful removal from the soil and minimal initial root damage. Plants (including soil block) were transported in large plastic bags.

2.2 Potting and water culture

To allow recovery of roots from handling during removal from the soil and transport, six plants were potted in soil in which they were dug out in the field.

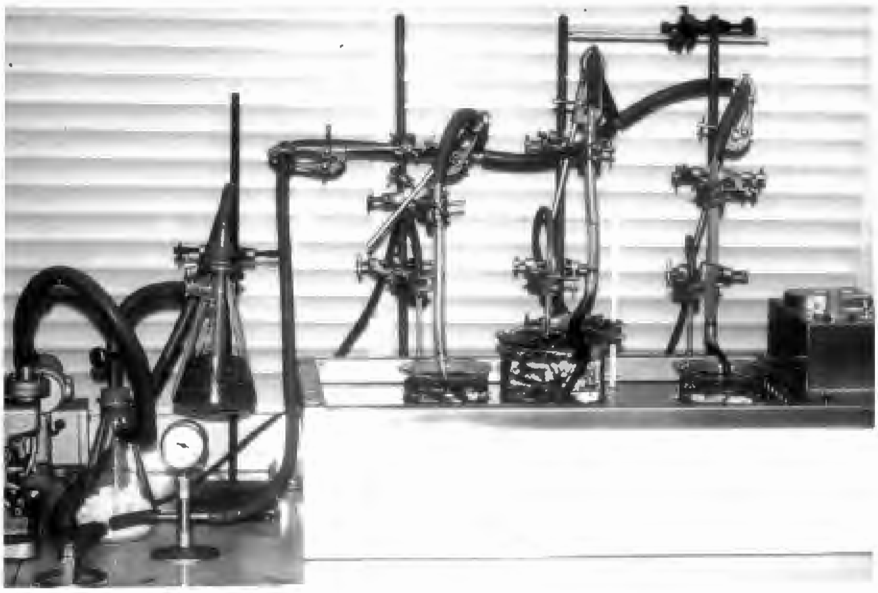
Three plants were also carefully washed free of soil and placed in a bucket containing ½ strength Hoagland solution which was continually aerated. The roots were kept dark by covering the bucket completely with aluminium foil. This was to prevent algal, fungal and other growths. Plants were kept at room temperature with adequate morning sunlight.

2.3 Plants used for the three experiments

Experiment 1: Three plants that had been potted for one week were used.

Experiment 2: Two water culture and one potted plant were used. The period of water culture and potting was 12 days.

Photograph 1: A single excised root of *Acacia cyclops* in an aerated nutrient feeding solution, attached to a vacuum system via the transparent plastic and opaque rubber tubes respectively.



Photograph 2: Experimental apparatus used for the investigation. A water bath containing three excised root systems attached to a mechanical vacuum pump system.

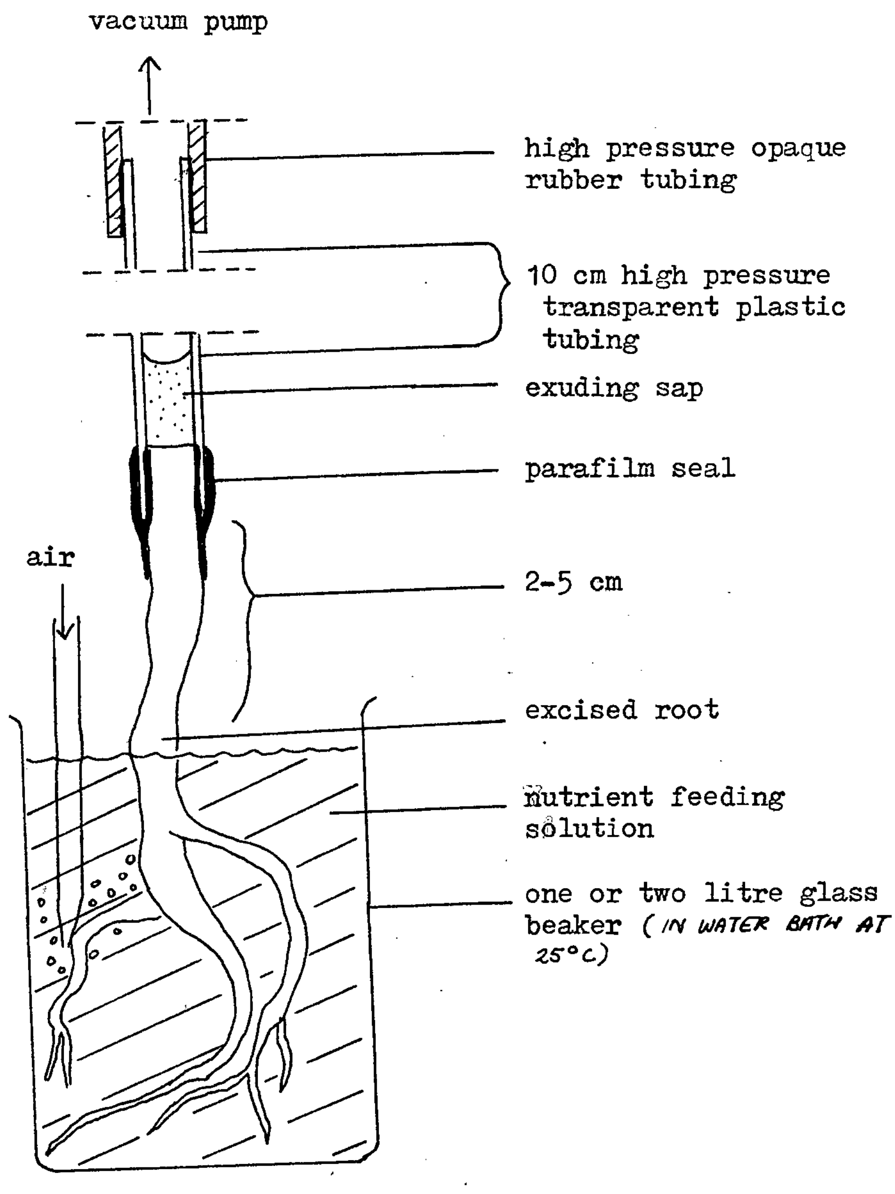


Figure 1: Diagram of the experimental system for a single excised root of *Acacia cyclops*.

(Two potted and one water culture plants died.)

Experiment 3: Four plants 'fresh' from the field were used.

2.4 Preparation for excision and erection of experimental apparatus

In all plants (except water culture), roots were carefully washed free of soil and excised 2-5cm above the first root branch. The time of excision was recorded together with the diameter of the stem at the point of excision. To calculate xylem vessel area, a transverse section of the stem was taken at this point. The area was calculated from photographs of these sections.

Paraflim was stretched firmly around the circumference of the stem just below the point of excision (see Figure 1 and Photograph 1). The root system was then attached to approximately 10cm transparent high pressure plastic tubing. Paraflim being again used to seal the tube-root junction as in figure 1. The transparent plastic tubing was then forced inside flexible opaque high pressure rubber tubing which was attached to a water vacuum pump (Experiments 1 and 2) or electrical pump (Experiment 3).

In Experiments 1 and 2 a pressure of -0,3 atm was used, while -1,0 atm was used in Experiment 3 (which had larger plants).

The root system was placed in a specific feeding solution in a one or two litre glass beaker, (depending on the size of the root system). This feeding solution was continually aerated and the beakers containing the root systems placed in a water bath at 25°C. These aeration and temperature conditions were found optimal for absorption of cations in Acacia cyclops and A. saligna (McKenzie, 1975).

2.5 Application of feeding solution and period of equilibrium

Roots were placed in feeding solutions containing high and low calcium and magnesium concentrations in four possible combinations as follows:

(a) Concentrations of calcium and magnesium:

	Ca (ppm)	Mg (ppm)
High	200	90
Low	20	20

The only other elements present in feeding solutions were Na (60 ppm) and K (7 ppm).

For comparison the calcium and magnesium concentrations used are similar to those used by McKenzie (1975) and are based on the exchangeable ion concentrations in the soils of the S.W. Cape (Flack 1975). The sodium and potassium concentrations are the average of the high and low concentrations he used.

(b) The following compounds were used:
(all Analar grade)

Calcium = $CaCl_2 6H_2O$

Magnesium = $MgSO_4.7H_2O$

Sodium = $NaNO_3$

Potassium = K_2SO_4

(c) The combinations of high and low concentrations of calcium and magnesium for the feeding solutions were:

1 = HCa + HMg
2 = HCa + LMg
3 = LCa + HMg
4 = LCa + LMg

TABLE 2.1: THE SEQUENCE OF APPLICATION OF FEEDING SOLUTIONS FOR THE RESPECTIVE PLANTS USED IN EXPERIMENTS 1, 2 AND 3

Plant ⟶	A	B	C	D*
Feeding solution concentration ratio (from above)	4 1 3 2	3 2 1 4	2 3 4 1	1 2 3 4

* (for Experiment 3 only)

The sequence was rerun for Experiment 3 only.

The condition of roots from the other experiments after the first run through the sequence did not warrant a rerun. The pH of all feeding solutions was noted.

Each concentration ratio was applied in a specific feeding solution for a period of 120 min. in Experiments 1 and 2, and 40 min. in Experiment 3 (at a higher pressure, with quantitatively more sap being collected as plants were larger - see Results). Time zero for each feeding solution application was when the vacuum pump was started.

Between each specific feeding solution application, an equilibrium period of two hours was established. The root systems were placed in distilled water (at 25°C in water bath and aerated), with the pump system not operating. This was to prevent carry-over of cations from one feeding solution to the next (see McKenzie, 1975).

2.6 Recording of sap flux

The rate of sap ascent in the transparent plastic tube was measured as from time zero (when the vacuum pump was started). Measurement was in millimetres at 10 min. intervals in Experiments 1 and 2 (120 min. application of feeding solution) and at 5 min. intervals for 40 min. in Experiment 3. (Measurement was terminated when the vacuum pump was stopped at the end of the feeding solution time.)

The volume of sap passing from the xylem per hour was calculated by application of linear regression. This was converted to flux by accounting for xylem area.

The effect of vacuum pressure on sap flux, using a ½ strength Hoagland feeding solution, was studied in Experiment 2 (Plant C).

In Experiment 3 sap flux was measured in a control run using ½ strength Hoagland feeding solution. The flux was measured when 10^{-4}, 10^{-3} and 10^{-2}M KCN was added to the feeding solution.

2.7 Collection and analysis of feeding solution and sap for calcium and magnesium concentration

5ml Feeding solution samples were taken at time zero, the start of each application.

Sap samples were taken at the end of each feeding solution application, just after the vacuum pump was switched off, (that is, at 120 min. in Experiments 1 and 2 and 40 min. in Experiment 3). The transparent plastic tumbe was disconnected from the rubber tube and sap was withdrawn by means of a syringe with a 2mm x 15cm teflon tube attached to its needle.

All samples were stored in glass bottles at 0^{o}C for approximately one week before analysis. Analysis was carried out on a Varian

Techton 1100 atomic absorption spectrophotometer.

2.8 Statistical analysis of the results

Sap flux values obtained for the two different plants in Experiment 3 control run were correlated with those obtained for different feeding solutions in Experiments 3(A) and 3(B). Control Run 1 was correlated with Experiment 3(A) and Control Run 2 with Experiment 3(B). The Spearman Rank correlation coefficient was used. In its simplest form this is:
(Snedecor and Cochran, 1967)

$$r_s = 1 - \frac{6 \sum d^2}{n(n^2 - 1)}$$

where n = the number of pairs being ranked
d = the horizontal difference between each pair.

3. RESULTS

TABLE 3.1 ANATOMICAL DIMENSIONS OF PLANTS USED FOR THE THREE EXPERIMENTS

(Measurements apply to point of excision)

Experiment and Plant		Stem Diameter (mm)	Total Xylem Area (mm^2)	Xylem Vessel Area (mm^2)
1	A	5,0	10,21	2,22
	B	3,0	2,95	0,78
	C	6,0	9,57	1,63
2	A	3,0	15,09	0,87
	B	3,5	15,39	1,70
	C	4,5	9,65	1,86
3	A	6,0	17,74	2,25
	B	7,5	48,50	6,64
	C	9,0	36,88	6,67
	D	10,0	77,75	11,72

TABLE 3.2: APPLICATION OF LINEAR REGRESSION TO MEASUREMENT OF SAP FLOW (ML MIN^{-1}) FOR THE DIFFERENT SETS OF EXPERIMENTS - (PLANTS + EXTERNAL FEEDING SOLUTION CONCENTRATION RATIO INDICATED)

(Expts 1 and 2 = Ml measurements at 20 min. intervals for 120 min)

(Expt 3 = Ml measurements at 5 min intervals for 40 min)

Characteristics of points described by ml against min for the plants at different conditions

Expt	Plant, Sol.Conc.Ratio	Y intercept	Slope (ml min) =Rate of Flow	r^2	Y Value at 60 min
1	A 1	0,048	0,019	0,998	1,163
	2	0,024	0,016	0,999	0,963
	3	0,075	0,013	0,992	0,826
	4	-0,040	0,017	0,998	1,006
	B 1	0,052	0,004	0,966	0,293
	2	0,046	0,007	0,983	0,443
	3	0,049	0,009	0,991	0,574
	4	0,021	0,004	0,983	0,279
	C 1	0,020	0,018	0,992	1,109
	2	-0,011	0,015	0,994	0,890
	3	0,062	0,017	0,996	1,070
	4	0,019	0,018	0,998	1,113
2	A 1	0,012	0,00036	0,842	0,033
	2	0,013	0,00045	0,873	0,040
	3	0,011	0,00054	0,926	0,043
	4	0,011	0,00054	0,920	0,044
	B 1	0,017	0,00048	0,829	0,046
	2	0,056	0,00051	0,915	0,043
	3	0,014	0,00051	0,875	0,045
	4	0,014	0,00057	0,896	0,048
	C 1	0,021	0,0021	0,985	0,148
	2	0,031	0,0022	0,969	0,165
	3	0,049	0,0030	0,956	0,228
	4	0,034	0,0021	0,955	0,162
					Y at 20 min
3(A)	A 1	0,024	0,022	0,996	0,455
	2	0,003	0,013	0,991	0,270
	3	0,030	0,014	0,990	0,312
	4	0,020	0,031	0,995	0,632
	B 1	0,075	0,062	0,996	1,322
	2	0,064	0,091	0,998	1,845
	3	0,160	0,096	0,994	1,972
	4	0,012	0,054	0,999	1,092
	C 1	-0,004	0,020	0,994	0,396
	2	0,153	0,017 (0.011)	0,678	0,389
	3	0,052	0,023	0,992	0,516
	4	0,021	0,021	0,995	0,459

	D	1	0,259	0,350	0,999	7,115
		2	0,123	0,160	0,999	3,317
		3	-0,048	0,094	0,997	1,826
		4	-0,050	0,074	0,998	1,436
3(B)	A	1	0,007	0,007	0,996	0,157
		2	0,012	0,008	0,997	0,171
		3	0,011	0,007	0,991	0,147
		4	0,014	0,009	0,993	0,198
	B	1	0,042	0,030	0,998	0,650
		2	-0,001	0,035	0,998	0,703
		3	0,045	0,037	0,997	0,783
		4	0,036	0,027	0,995	0,572
	C	1	0,024	0,013	0,988	0,282
		2	0,030	0,013	0,988	0,298
		3	0,014	0,014	0,996	0,298
		4	0,016	0,012	0,994	0,262
	D	1	-0,023	0,056	0,999	1,090
		2	0,009	0,051	0,999	1,011
		3	-0,038	0,048	0,997	0,922
		4	-0,006	0,045	0,997	0,884
Control	1	A	0,005	0,017	0,997	0,353
		B	0,039	0,075	0,997	1,537
		C	0,012	0,020	0,998	0,417
		D	0,057	0,117	0,999	2,400
	2	A	0,009	0,009	0,983	0,197
		B	0,023	0,035	0,997	0,770
		C	0,014	0,014	0,996	0,290
		D	-0,059	0,052	0,997	0,983

TABLE 3.3: SAP FLUX MEASUREMENTS FOR PLANTS SUBJECTED TO DIFFERENT FEEDING SOLUTION CONCENTRATIONS IN THE THREE EXPERIMENTS.

(Rate from linear regression, Area = Vessel area)

			Sap Flux (ml hr^{-1} mm^{-2})				
Feeding Conc.⟶			HCa HMg	HCa LMg	LCa HMg	LCa LMg	
Expt,	Plant.	Diameter (mm)					Mean
1	A	5,0	0,51	0,43	0,35	0,46	
	B	3,0	0,31	0,54	0,69	0,31	
	C	6,0	0,66	0,55	0,62	0,66	
2	A	3,0	0,025	0,025	0,037	0,037	
	B	3,5	0,017	0,018	0,018	0,020	
	C	4,5	0,069	0,072	0,097	0,069	
3(A)	A	6,0	0,59	0,35	0,37	0,83	.53
	B	7,5	0,56	0,82	0,87	0,49	.685
	C	9,0	0,18	0,15	0,21	0,19	.182
	D	10,0	1,79?	0,82	0,48	0,38	.96
3(B)	A	6,0	0,19	0,21	0,19	0,24	.207
	B	7,5	0,27	0,32	0,33	0,24	.296
	C	9,0	0,12	0,12	0,13	0,11	.120
	D	10,0	0,29	0,26	0,25	0,23	.257
3(Avg)	A	6,0	0,39	0,28	0,28	0,54	
	B	7,5	0,42	0,57	0,60	0,37	
	C	9,0	0,15	0,14	0,17	0,15	
	D	10,0	1,04?	0,54	0,37	0,31	

TABLE 3.4: SAP FLUX MEASUREMENTS FOR THE CONTROL RUN IN EXPERIMENT 3. (Rate from linear regression, Area = Vessel area)

		Sap Flux (ml hr^{-1} mm^{-2})	
Run →		1	2
Plant	Diameter (mm)		
A	6,0	0,46	0,24
B	7,5	0,68	0,32
C	9,0	0,18	0,13
D	10,0	0,60	0,27

external soln & Hoagland's ?

TABLE 3.5: THE EFFECT OF VACUUM PRESSURE ON SAP FLUX - MEASUREMENTS USING PLANT C FROM EXPT. 2.

(Feeding Solution = ½ Str Hoagland)
(Each Pressure = 30 min run)

Pressure (ATM)	Sap Flux ($ml\ hr^{-1}\ mm^{-2}$) x 10^{-3} (Average of 2 runs)	
0	0,21	(Measured with zero pressure over 8h30)
-0,3	0,45	
-0,6	0,54	
-1,0	0,67	
-1,3	0,86	

?

TABLE 3.6: EFFECT OF CYANIDE ON SAP FLUX:

(EXPERIMENT 3)

(Pressure = -1ATM, Feeding Solution = ½ strength Hoagland, Run = 15 min at each cyanide conc.)

Cyanide Concentration M	Plant, Flux (ml hr^{-1} mm^{-2})			
	A	B	C	D
Control (O)	0,169	0,096	0,057	0,160
10^{-4}	0,133	0,077	0,037	0,142
10^{-3}	0,102	0,154	0,037	0,177
10^{-2}	0,102	0,134	0,076	0,354

TABLE 3.7: CATION CONCENTRATION OF FEEDING SOLUTIONS AND XYLEM SAP FOR DIFFERENT EXPERIMENTS, PLANTS AND FEEDING SOLUTION CONCENTRATION RATIOS.

Expt, Plants, Sol.Conc. Ratio	Feeding Solution Concentration (ppm) Ca	Mg	Sap Concentration (ppm) Ca	Mg
1 A 1	159	97	103	82
2	214	22	103	49
3	20	83	62	62
4	17	23	34	24
B 1	159	90	62	21
2	207	29	62	17
3	28	78	76	23
4	14	24	69	17
C 1	159	97	79	66
2	221	24	117	48
3	28	76	55	40
4	14	24	28	20
2 A 1	172	95	62	-
2	200	24	62	-
3	20	73	34	-
4	17	25	20	-
B 1	145	89	69	-
2	207	24	83	-
3	30	76	48	-
4	20	23	28	-
C 1	166	92	110	57
2	207	30	103	48
3	28	78	76	38
4	14	23	76	54

Expt, Plant, Sol.Conc. Ratio	Feeding Solution Concentration (ppm)				Sap Concentration (ppm)			
	Ca		Mg		Ca		Mg	
	A*	B*	A	B	A	B	A	B
3(A)(B) A1	142	146	107	117	42	56	23	12
2	212	188	28	22	31	28	14	12
3	21	21	88	91	28	31	10	21
4	14	14	26	25	18	42	14	15
B1	131	131	95	95	42	42	28	29
2	196	174	26	27	70	35	24	14
3	21	21	75	84	35	28	20	24
4	14	14	26	27	35	21	19	14
C1	131	131	105	102	42	28	22	23
2	204	174	29	28	42	31	19	23
3	21	21	67	87	28	21	16	18
4	14	21	26	29	14	21	19	10
D1	131	138	102	99	97?	21	73?	30
2	196	181	28	27	76	20	29	22
3	21	18	88	87	21	17	22	19
4	30	14	14	27	16	13	21	14

(A = Expt.3A, B = Expt.3B)

TABLE 3.8: TIME OF FEEDING SOLUTION APPLICATION IN EXPERIMENTS 3(A) AND 3(B)

	Hours after detopping (hr)			
Feeding Sol.→	(1) HCa HMg	(2) HCa LMg	(3) LCa HMg	(4) LCa LMg
Expt. and Plant				
3(A) A	98.15	121.15	115.45	92.15
B	116.30	99.00	93.00	122.00
C	121.00	92.00	98.00	121.00
D	94.30	100.30	118.00	123.30
3(B) A	144.15	150.15	147.35	140.15
B	148.20	145.00	141.00	151.00
C	150.00	140.00	144.00	150.00
D	142.30	146.30	149.50	152.30

TABLE 3.9: CORRELATION OF CONTROL RUN SAP FLUX VALUES FOR EXPERIMENT 3 WITH THE SAP FLUX VALUES OBTAINED FOR DIFFERENT FEEDING SOLUTIONS IN EXPERIMENTS 3(A) AND 3(B).

Control Run 1* is correlated with Experiment 3(A) and Control Run 2* with Experiment 3(B) using the Spearman Rank correlation coefficient. (Values for plants A,B,C and D ranked)

Expt.	Feeding Solution	r_s	Significant or not (2 d.f. at 1% level)
3(A)	HCa HMg	0,8553	NS
	HCa LMg	0,9903	S
	LCa HMg	0,9919	S
	LCa LMg	0,9779	NS
3(B)	HCa HMg	0,9995	S
	HCa LMg	0,9999	S
	LCa HMg	0,9997	S
	LCa LMg	0,9992	S

(Note: Control Run 1 = 90 hrs after detopping
Run 2 = 155 hrs after detopping)

Do not understand of Tables 3.3 & 3.4.

TABLE 3.10: RANKING OF ROOT FEEDING SOLUTIONS (VARYING Ca AND Mg CONCENTRATIONS) AS ASSOCIATED WITH THE HIGHEST TO LOWEST SAP FLUX, SAP (Ca) AND SAP (Mg) FOR THE DIFFERENT PLANTS IN THE THREE EXPERIMENTS (i.e. Summary of Tables and Graphs)

Feeding Solutions:
High Ca + High Mg = 1
High Ca + Low Mg = 2
Low Ca + High Mg = 3
Low Ca + Low Mg = 4

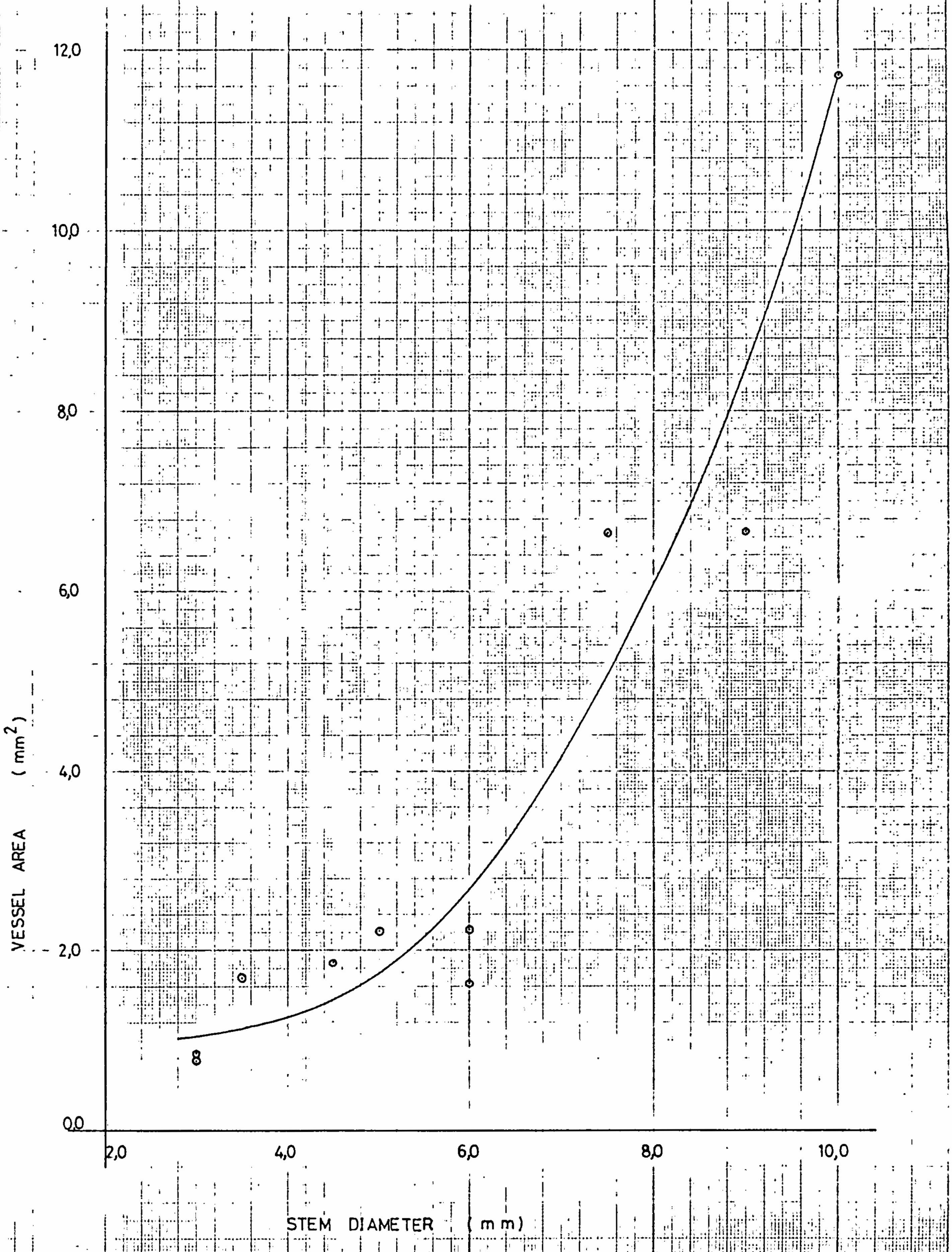
Highest to lowest sap flux, sap (Ca) and sap (Mg) rank for a particular plant in each experiment = (a) (d).
(Brackets = same value)

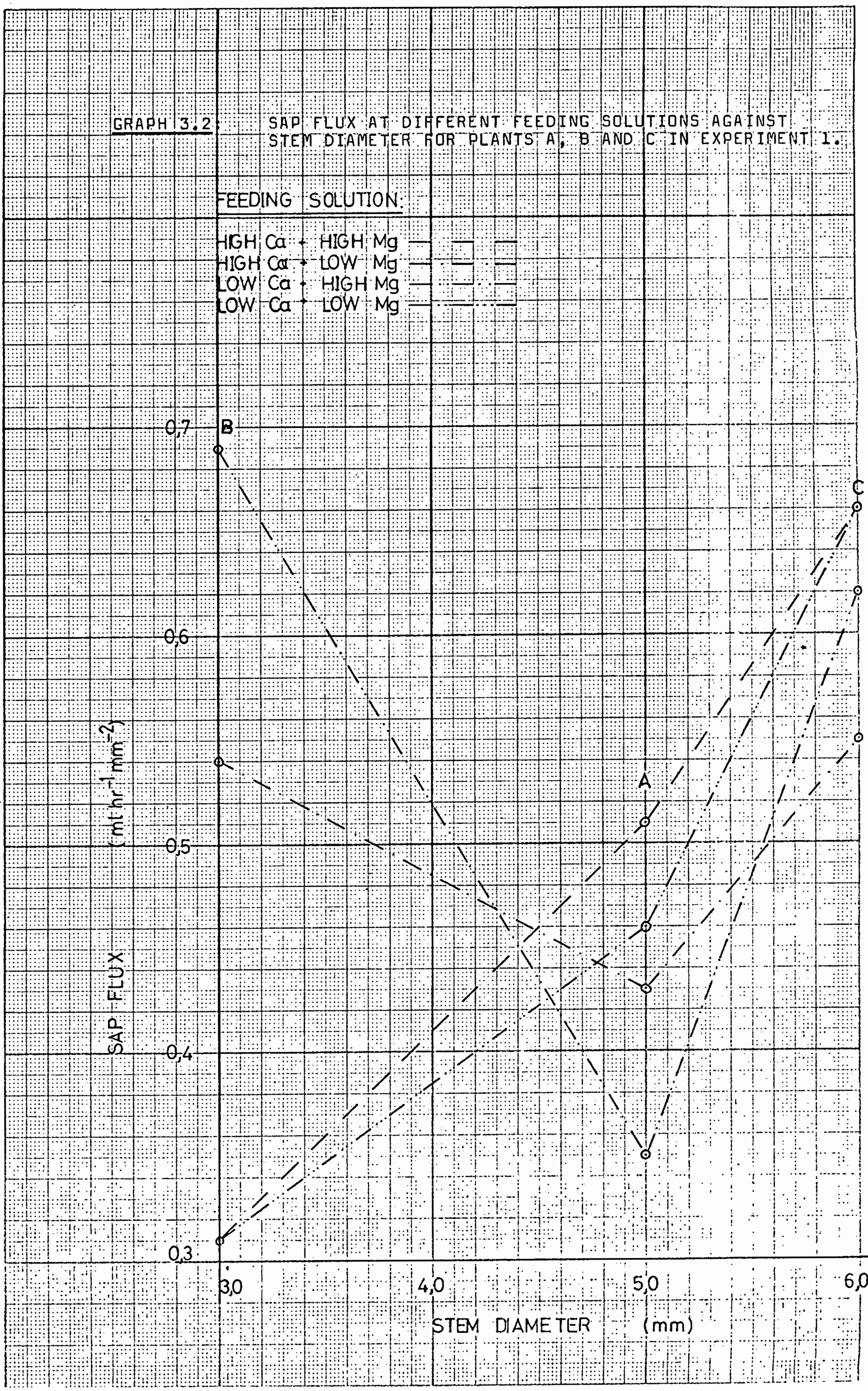
Plant Expt and High to Low Rank		FEEDING SOLUTION											
		A			B			C			D		
		Flux	Ca	Mg	Flux	Ca	Mg	Flux	Ca	Mg	Flux	Ca	Mg
1	(a)	xx1	1	1	xx3	3	3	xx1	2	1		-	
	(b)	4	2	3	2	4	1	4	1	2		-	
	(c)	2	3	2	1	1	2	3	3	3		-	
	(d)	3	4	4	4	2	4	2	4	4		-	
2	(a)	✓3	1	-	x4	2	-	xx3	1	1		-	
	(b)	4	2	-	2	1	-	2	2	4		-	
	(c)	1	3	-	3	3	-	1	3	2		-	
	(d)	2	4	-	1	4	-	4	4	3		-	
3(A)	(a)	4	1	1	3	2	1	3	1	1	1	1	1
	(b)	1	2	2	2	1	2	4	2	2	2	2	2
	(c)	3	3	4	1	3	3	1	3	4	3	3	3
	(d)	2	4	3	4	4	4	2	4	3	4	4	4
3(B)	(a)	4	1	3	3	1	1	3	2	1	1	1	1
	(b)	3	4	4	2	2	2	1	1	2	2	2	2
	(c)	1	3	1	1	3	4	2	3	3	3	3	3
	(d)	2	2	2	4	4	3	4	4	4	4	4	4

x water culture
xx Potted

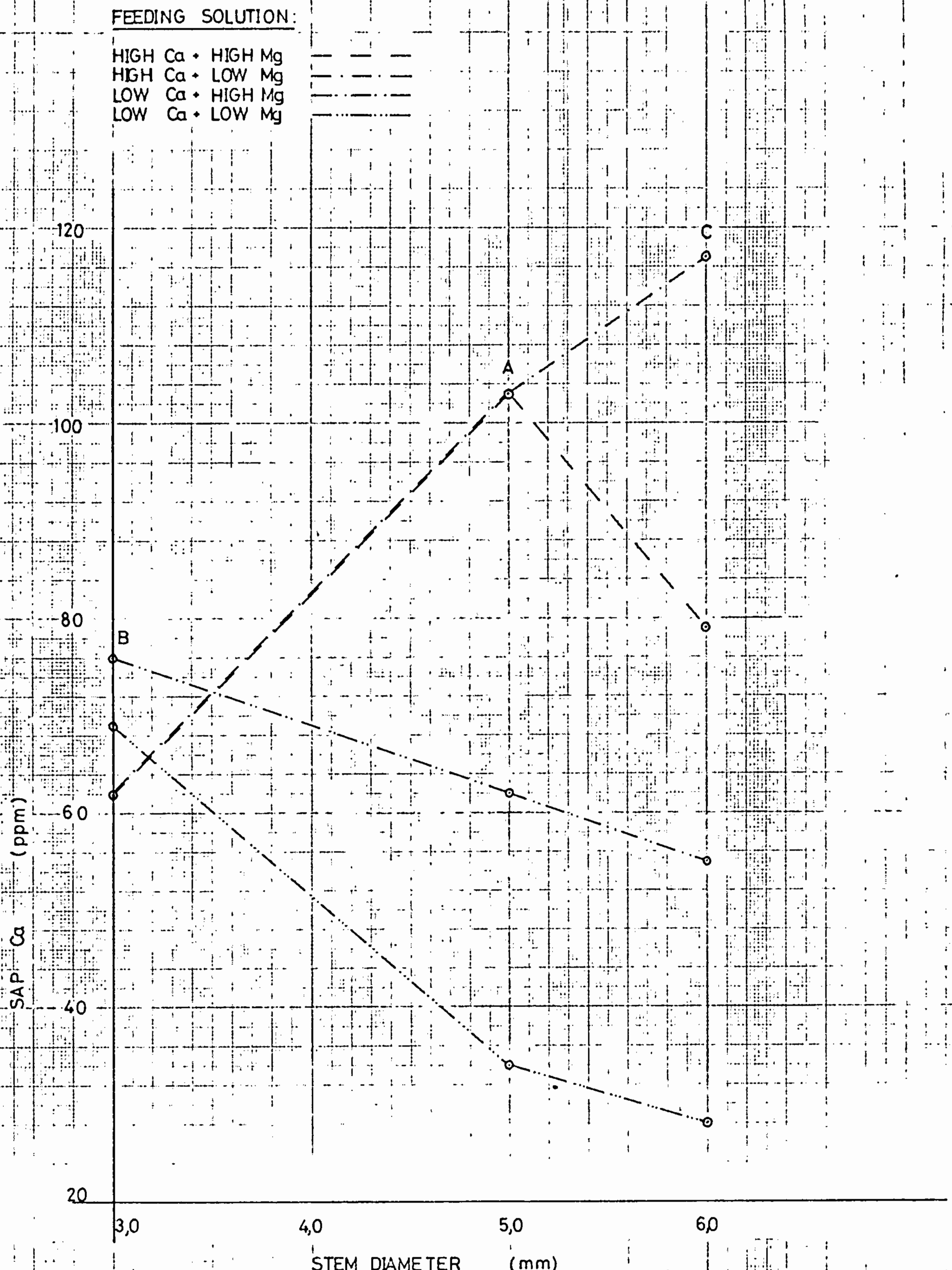
168 exper. investigation

GRAPH 3.1: VESSEL AREA AGAINST STEM DIAMETER FOR ALL PLANTS USED IN EXPERIMENTS 1, 2 AND 3.

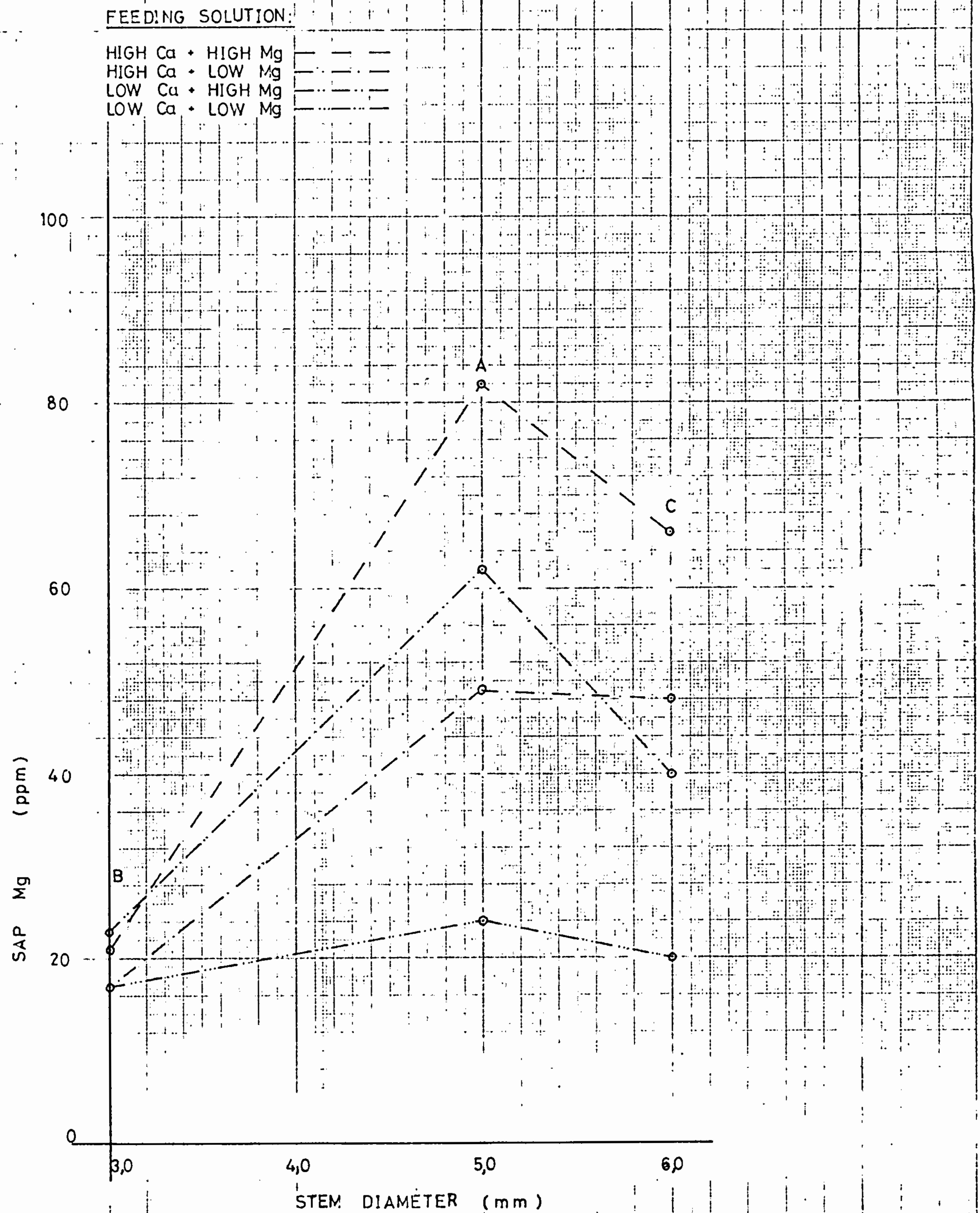



GRAPH 3.2 SAP FLUX AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B AND C IN EXPERIMENT 1.
FEEDING SOLUTION:
HIGH Ca + HIGH Mg
HIGH Ca + LOW Mg
LOW Ca + HIGH Mg
LOW Ca + LOW Mg
SAP FLUX (ml hr^{-1} mm^{-2})
0,7
0,6
0,5
0,4
0,3
B
A
C
3,0
4,0
5,0
6,0
STEM DIAMETER (mm)

GRAPH 3.3: SAP CALCIUM CONCENTRATION AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B AND C IN EXPERIMENT 1.

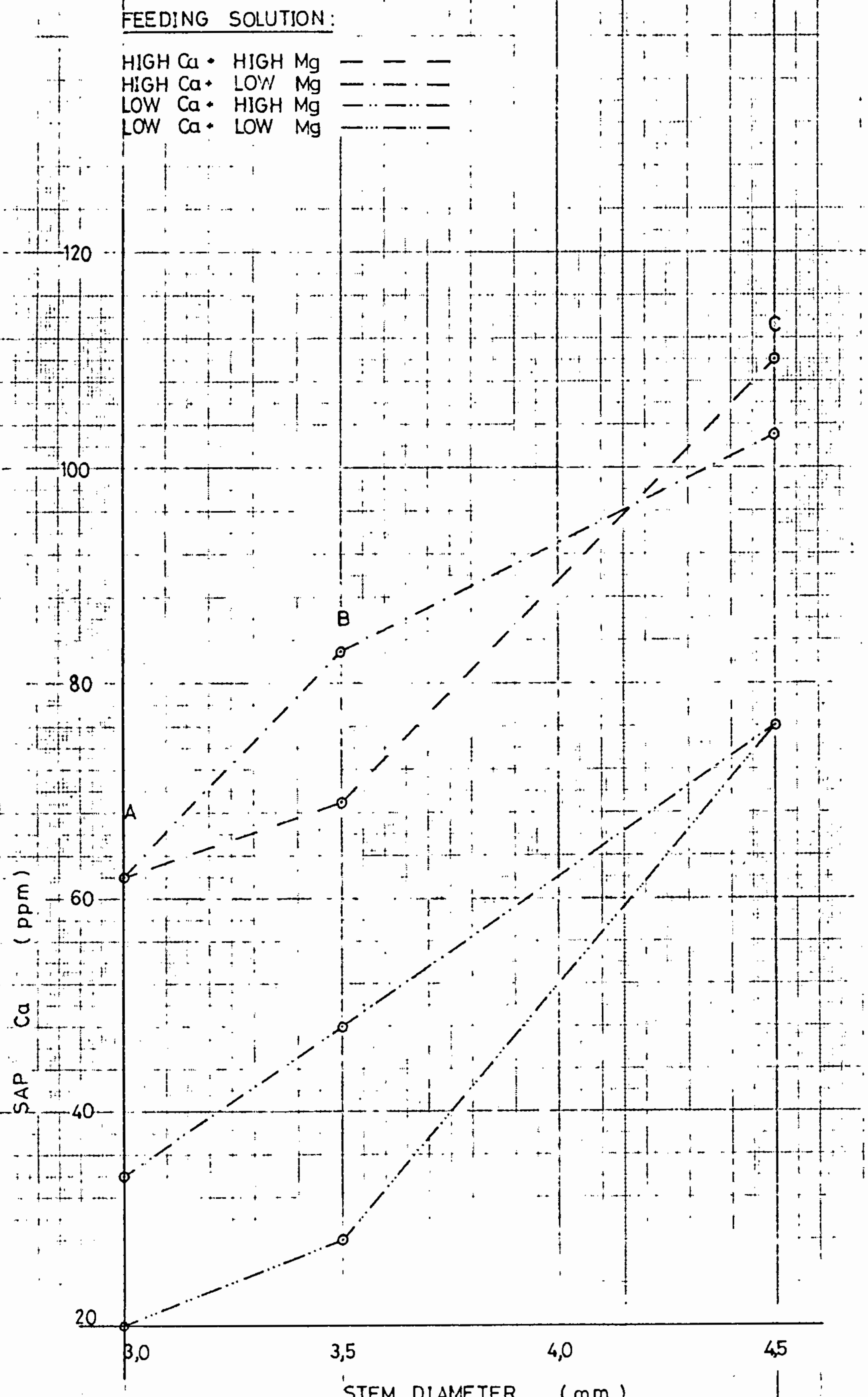


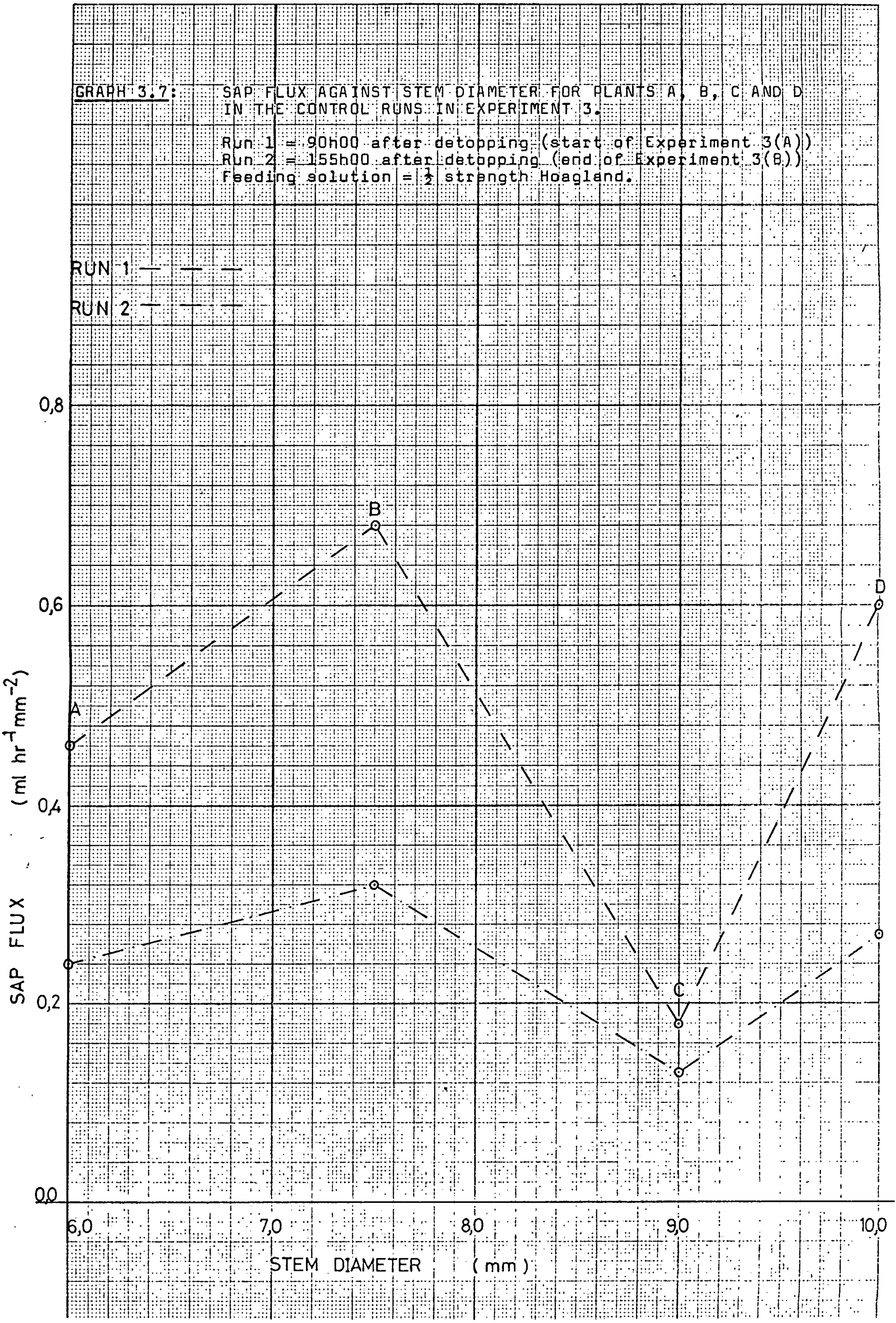
GRAPH 3.4: SAP MAGNESIUM CONCENTRATION AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B AND C IN EXPERIMENT 1.



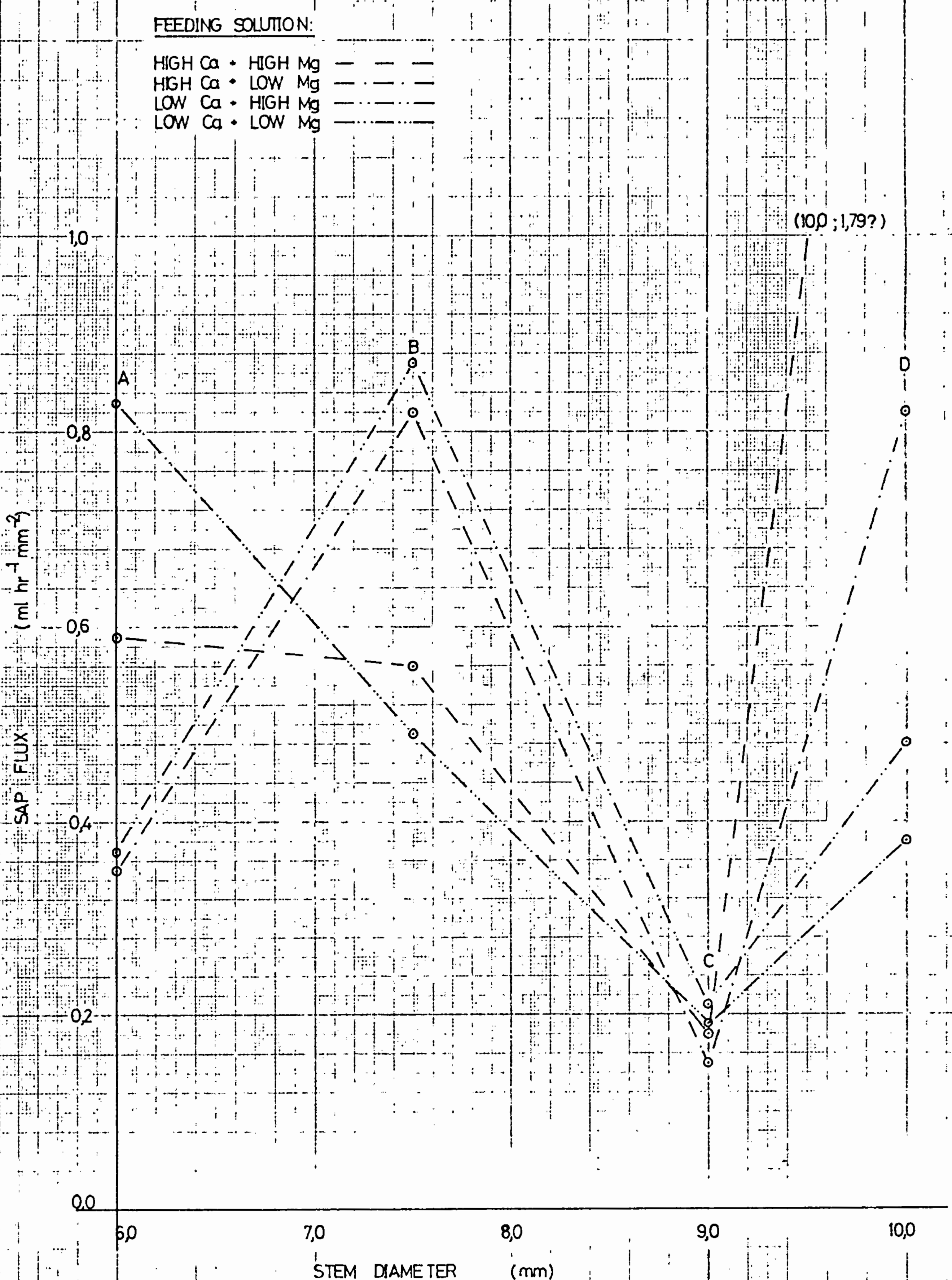

GRAPH 3.5: SAP FLUX AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B AND C IN EXPERIMENT 2.
FEEDING SOLUTION:
HIGH Ca + HIGH Mg
HIGH Ca + LOW Mg
LOW Ca + HIGH Mg
LOW Ca + LOW Mg
0,10
0,08
0,06
0,04
0,02
0,00
A
B
C
SAP FLUX (ml hr-1 mm-2)
3,0
3,5
4,0
4,5
STEM DIAMETER (mm)

GRAPH 3.6: SAP CALCIUM CONCENTRATION AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B AND C IN EXPERIMENT 2.

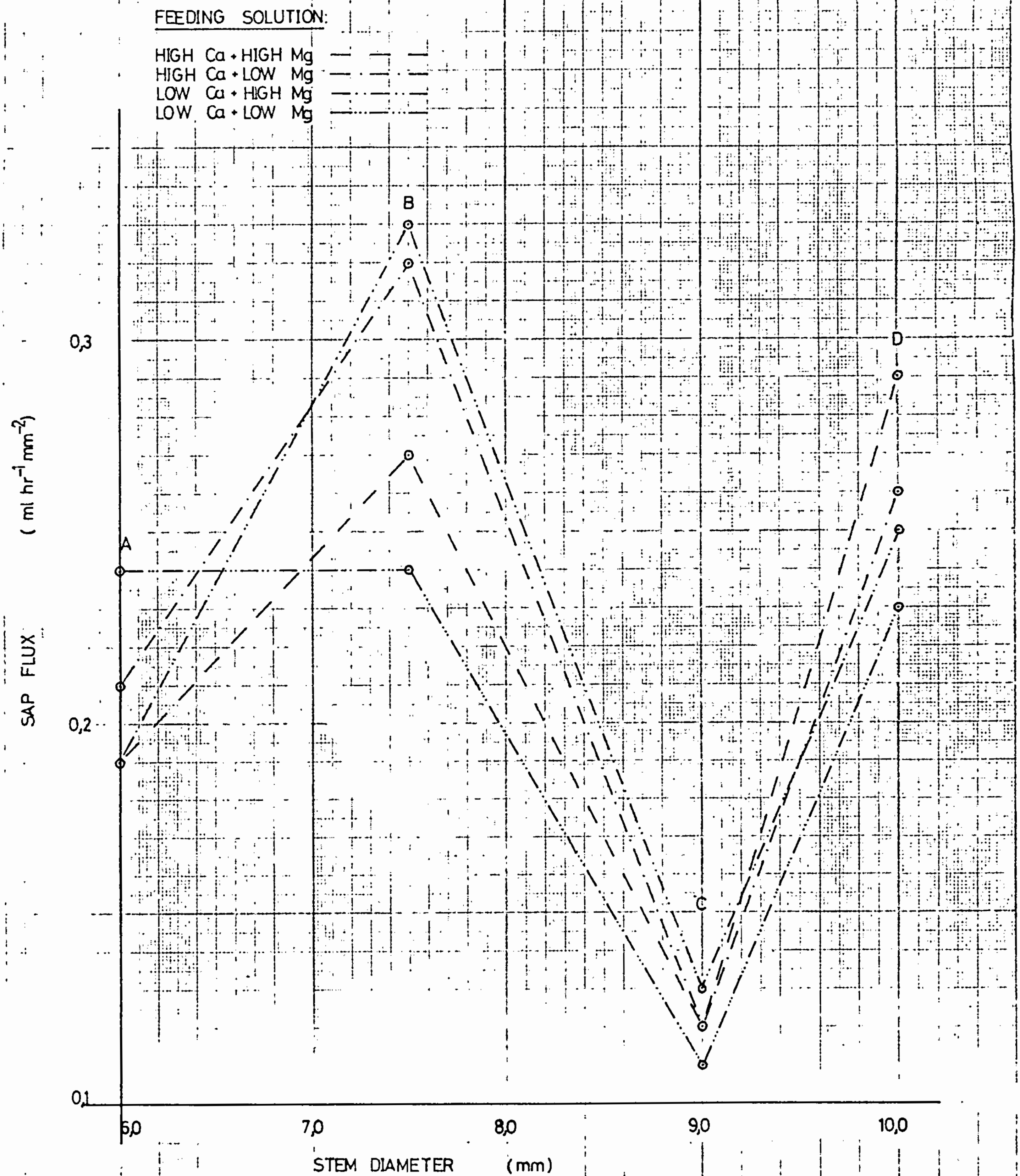



GRAPH 3.7: SAP FLUX AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN THE CONTROL RUNS IN EXPERIMENT 3.
Run 1 = 90h00 after detopping (start of Experiment 3(A))
Run 2 = 155h00 after detopping (end of Experiment 3(B))
Feeding solution = ½ strength Hoagland.
RUN 1
RUN 2
A
B
C
D
0,0
0,2
0,4
0,6
0,8
SAP FLUX (ml hr⁻¹ mm⁻²)
6,0
7,0
8,0
9,0
10,0
STEM DIAMETER (mm)

GRAPH 3.8: SAP FLUX AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3(A).

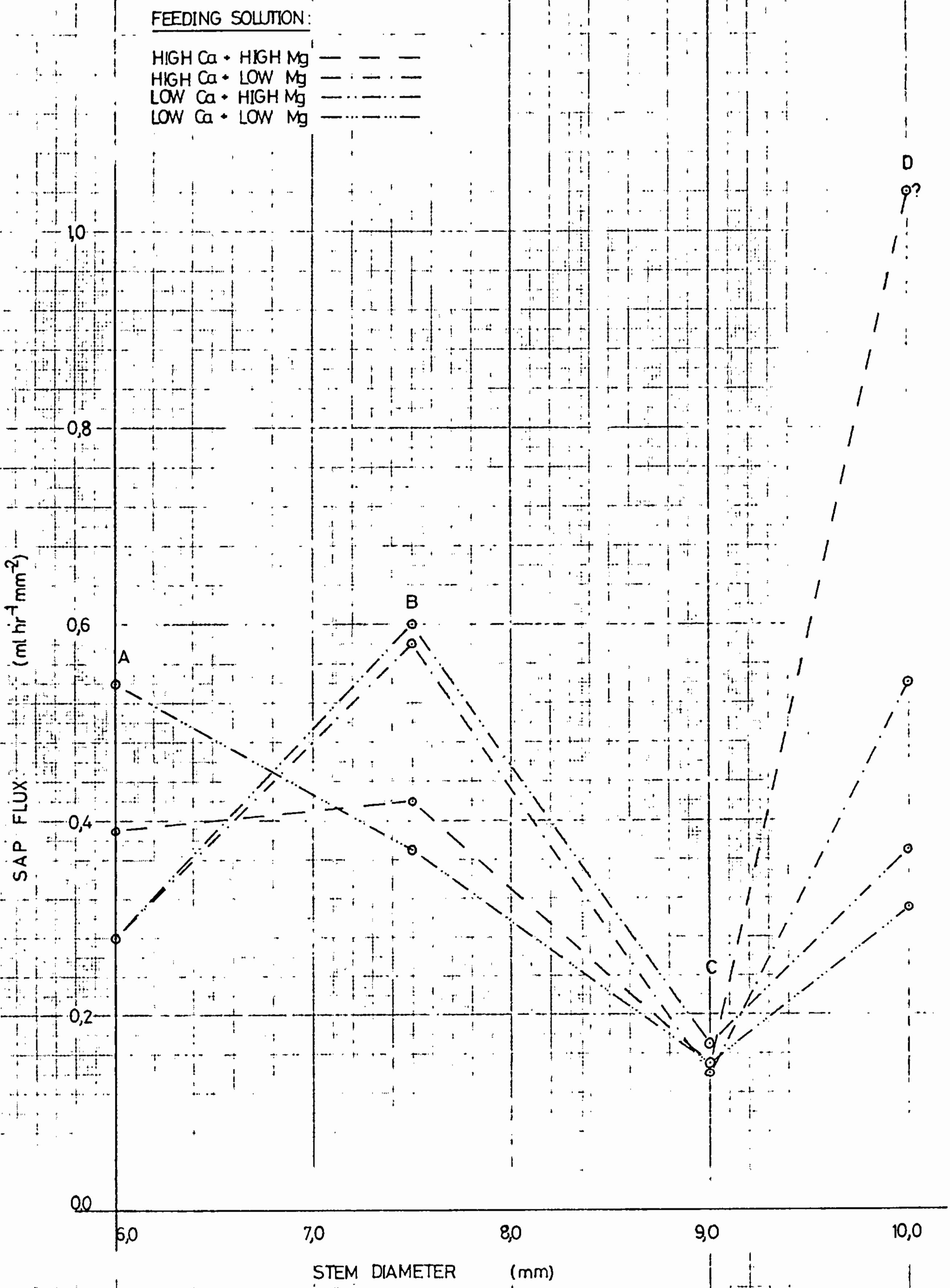


GRAPH 3.9: SAP FLUX AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3(B).



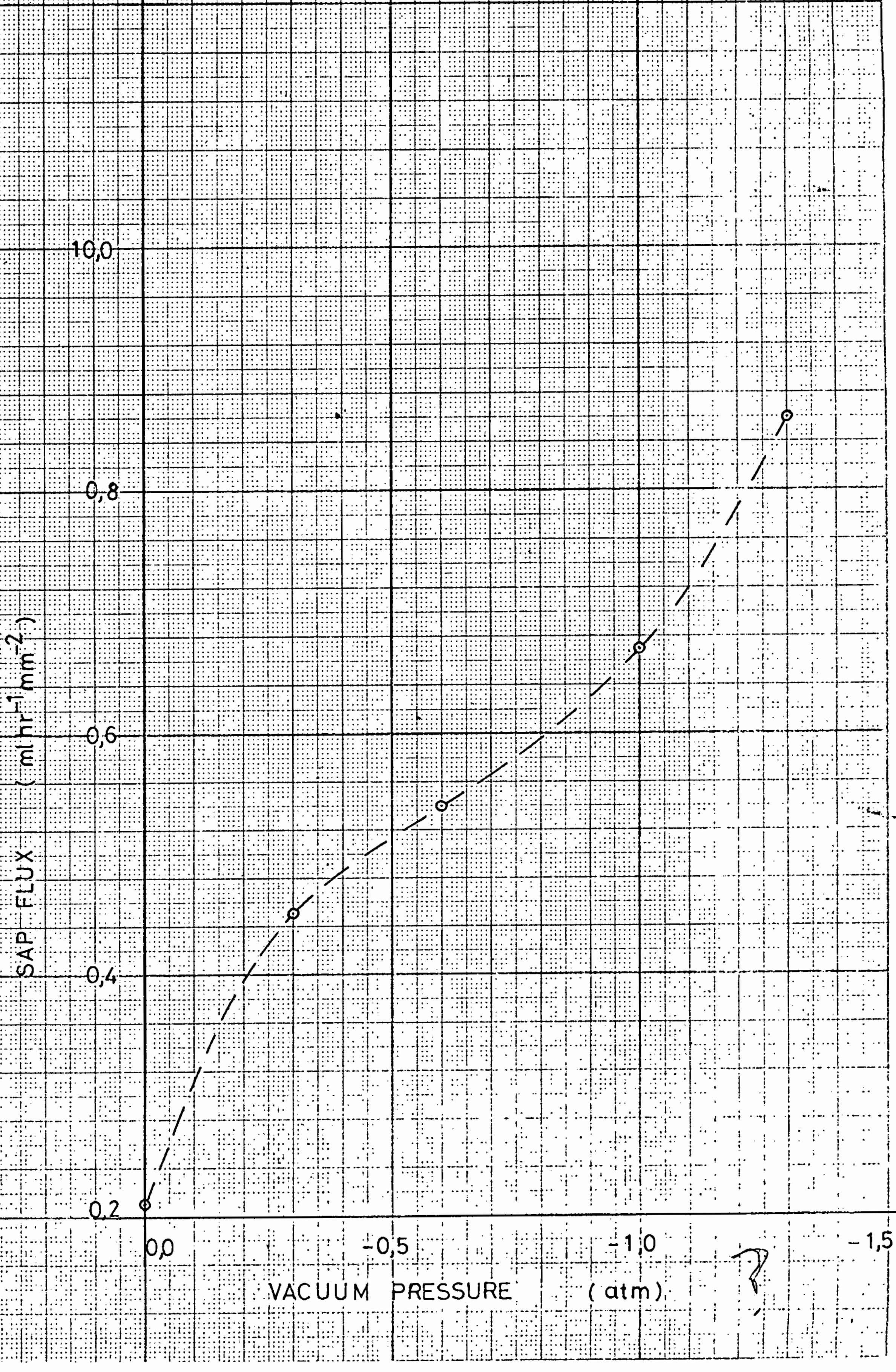
W831 Waltons Stationery Co. (Pty) Ltd.

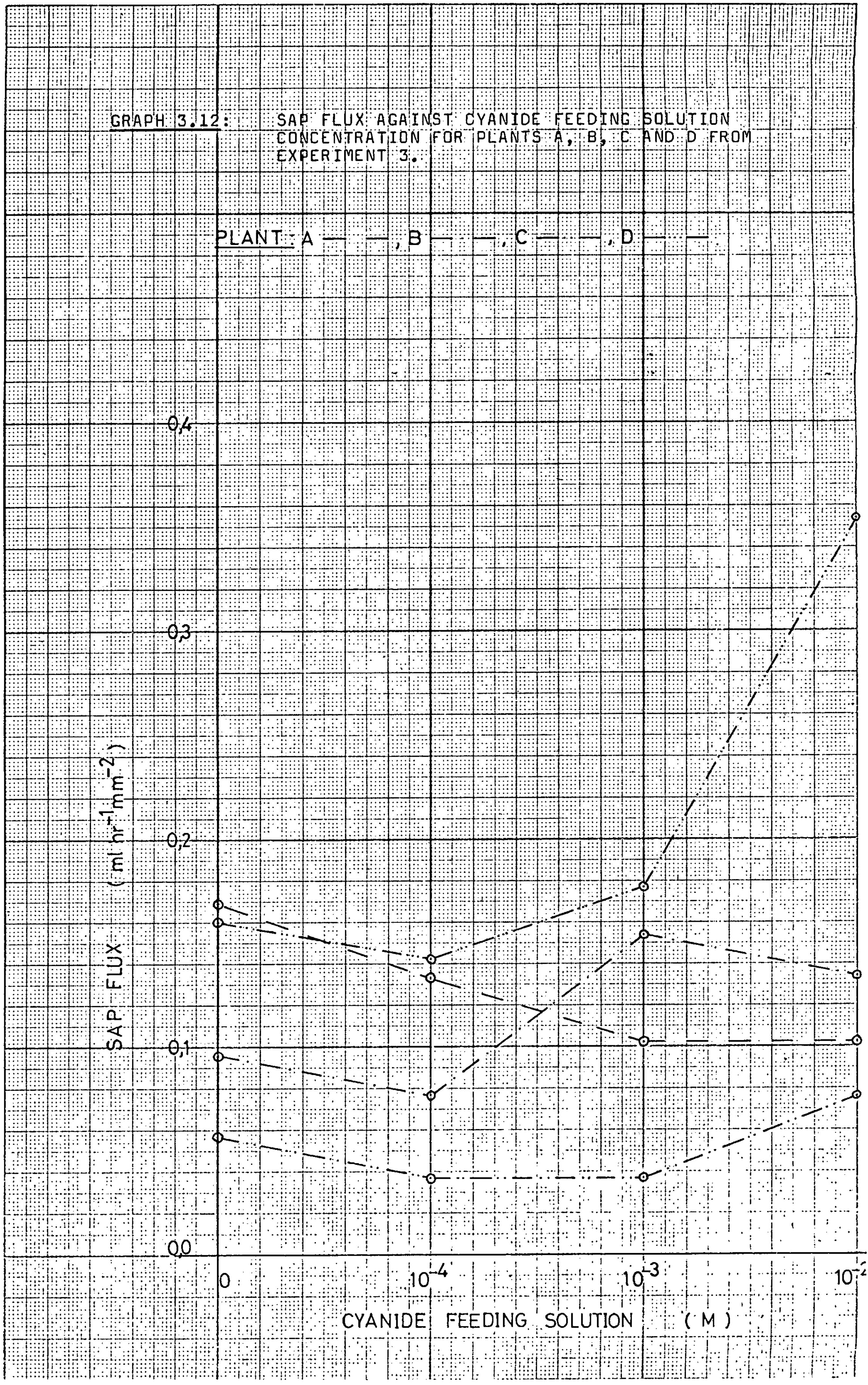
GRAPH 3.10: AVERAGE SAP FLUX AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, C, C AND D IN EXPERIMENTS 3(A) AND (B).



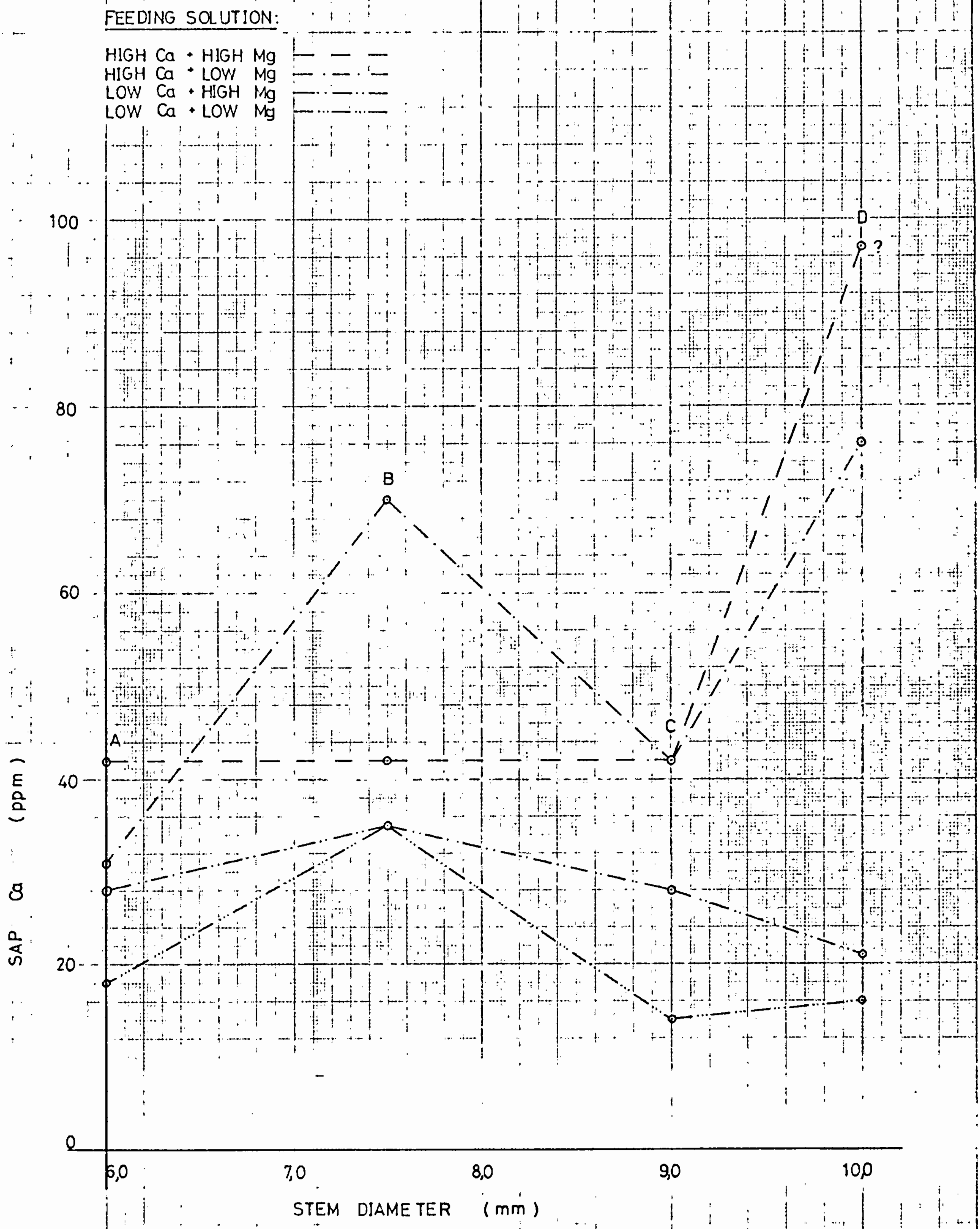
GRAPH 3.11: SAP FLUX AGAINST VACUUM PRESSURE FOR PLANT C IN EXPERIMENT 2.

Average flux value for 2 x 30 min runs with ½ strength Hoagland feeding solution.

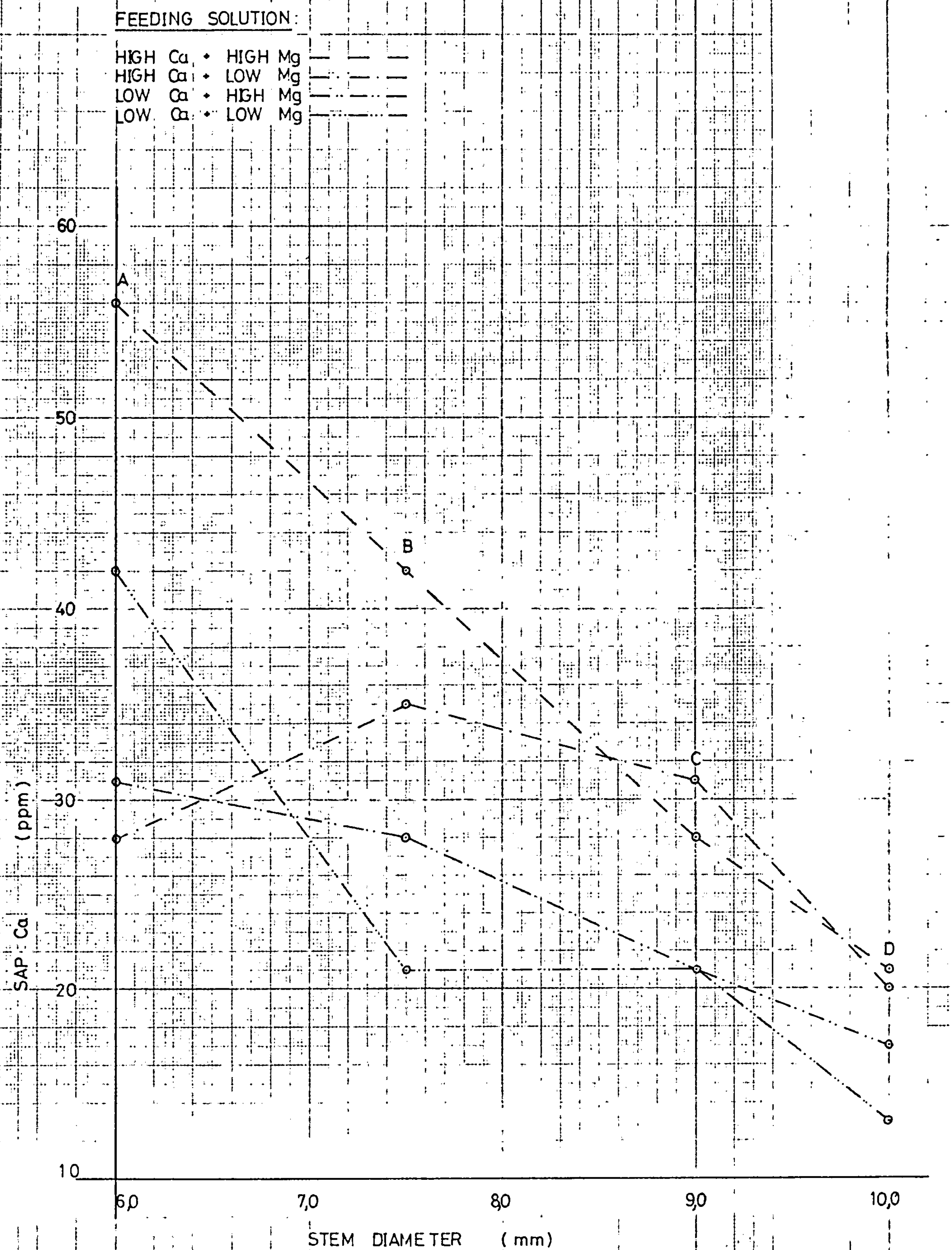



GRAPH 3.12: SAP FLUX AGAINST CYANIDE FEEDING SOLUTION CONCENTRATION FOR PLANTS A, B, C AND D FROM EXPERIMENT 3.
PLANT : A, B, C, D
SAP FLUX (ml hr-1 mm-2)
0,4
0,3
0,2
0,1
0,0
0
10-4
10-3
10-2
CYANIDE FEEDING SOLUTION (M)

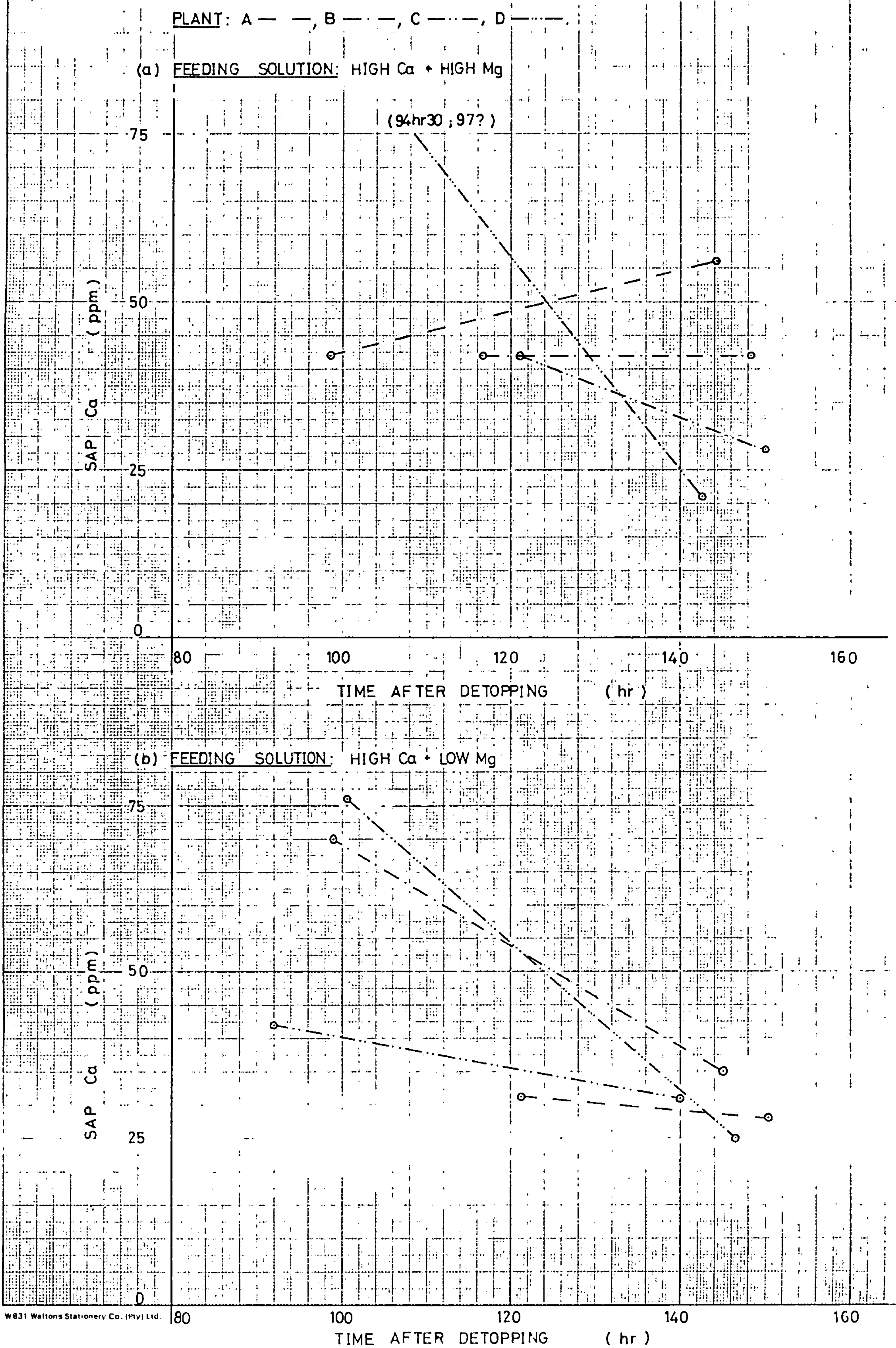
GRAPH 3.13: SAP CALCIUM CONCENTRATION AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3(A).



GRAPH 3.14: SAP CALCIUM CONCENTRATION AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3(B).



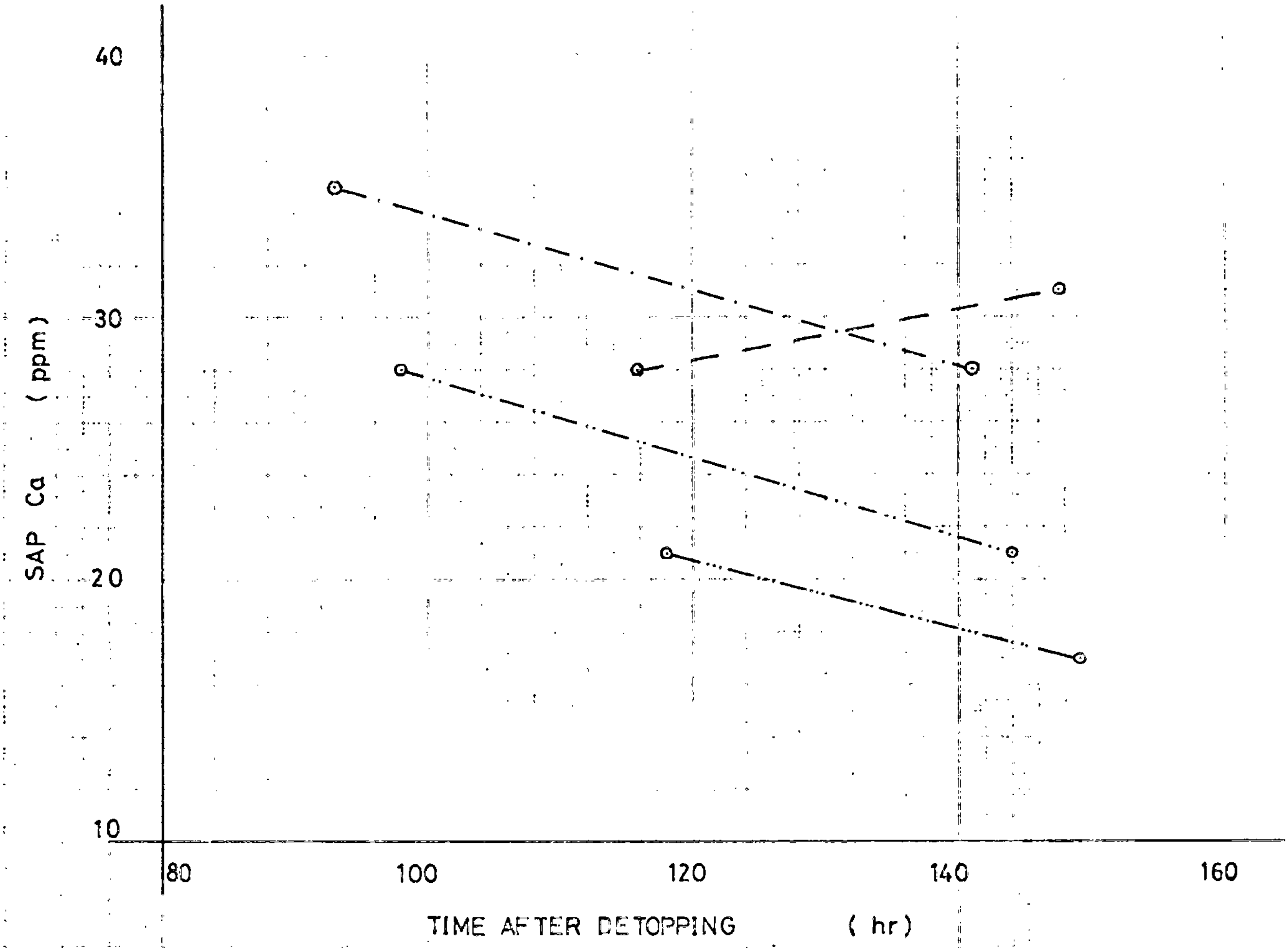
GRAPH 3,15(a): SAP CALCIUM CONCENTRATION AT SPECIFIC FEEDING SOLUTIONS AGAINST TIME AFTER DETOPPING FOR PLANTS A, B, C AND D IN EXPERIMENT 3.



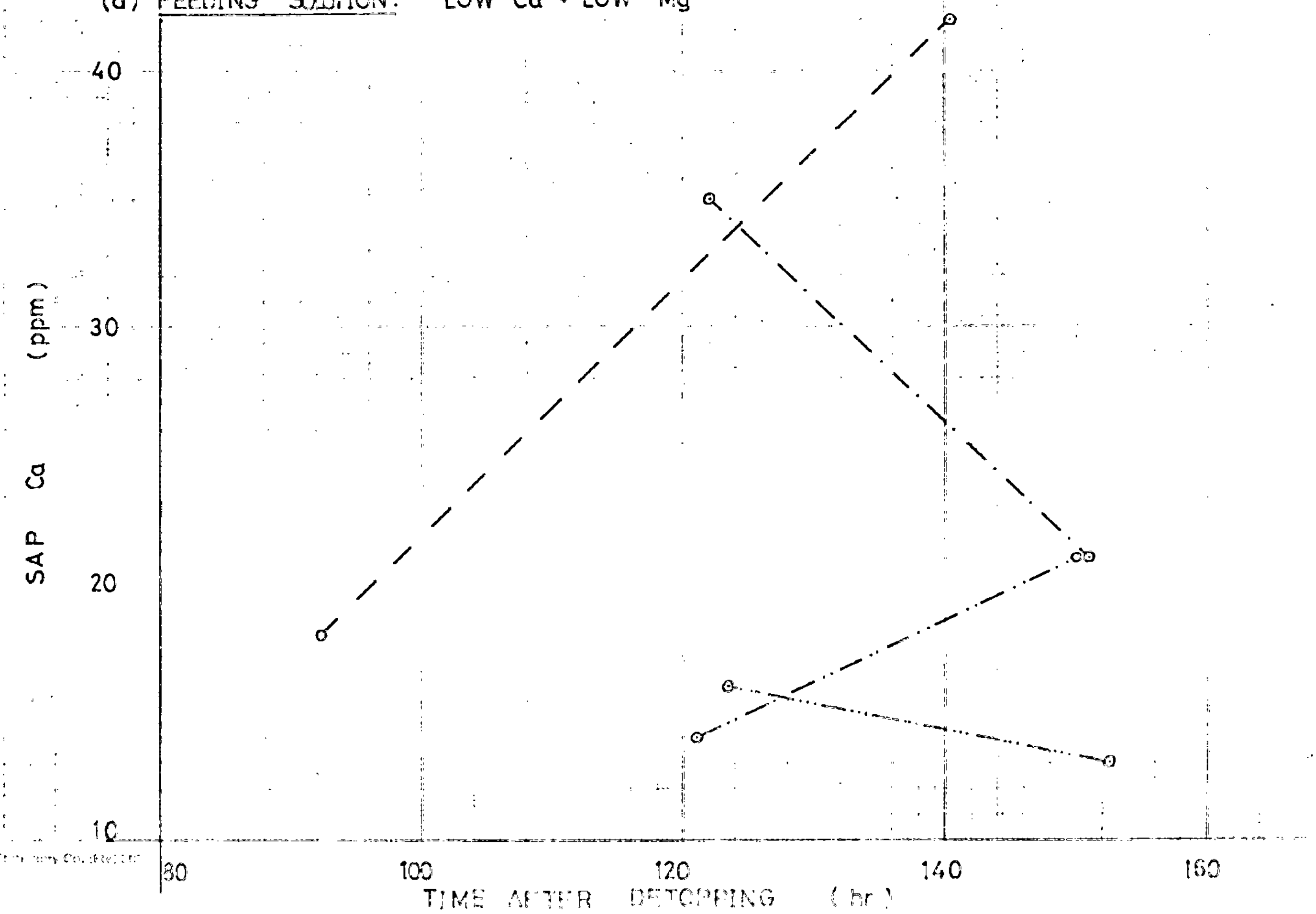
GRAPH 3.15(b): SAP CALCIUM CONCENTRATION AT SPECIFIC FEEDING SOLUTIONS AGAINST TIME AFTER DETOPPING FOR PLANTS A, B, C AND D IN EXPERIMENT 3.

PLANT: A — —, B —·—, C —··—, D —···—.

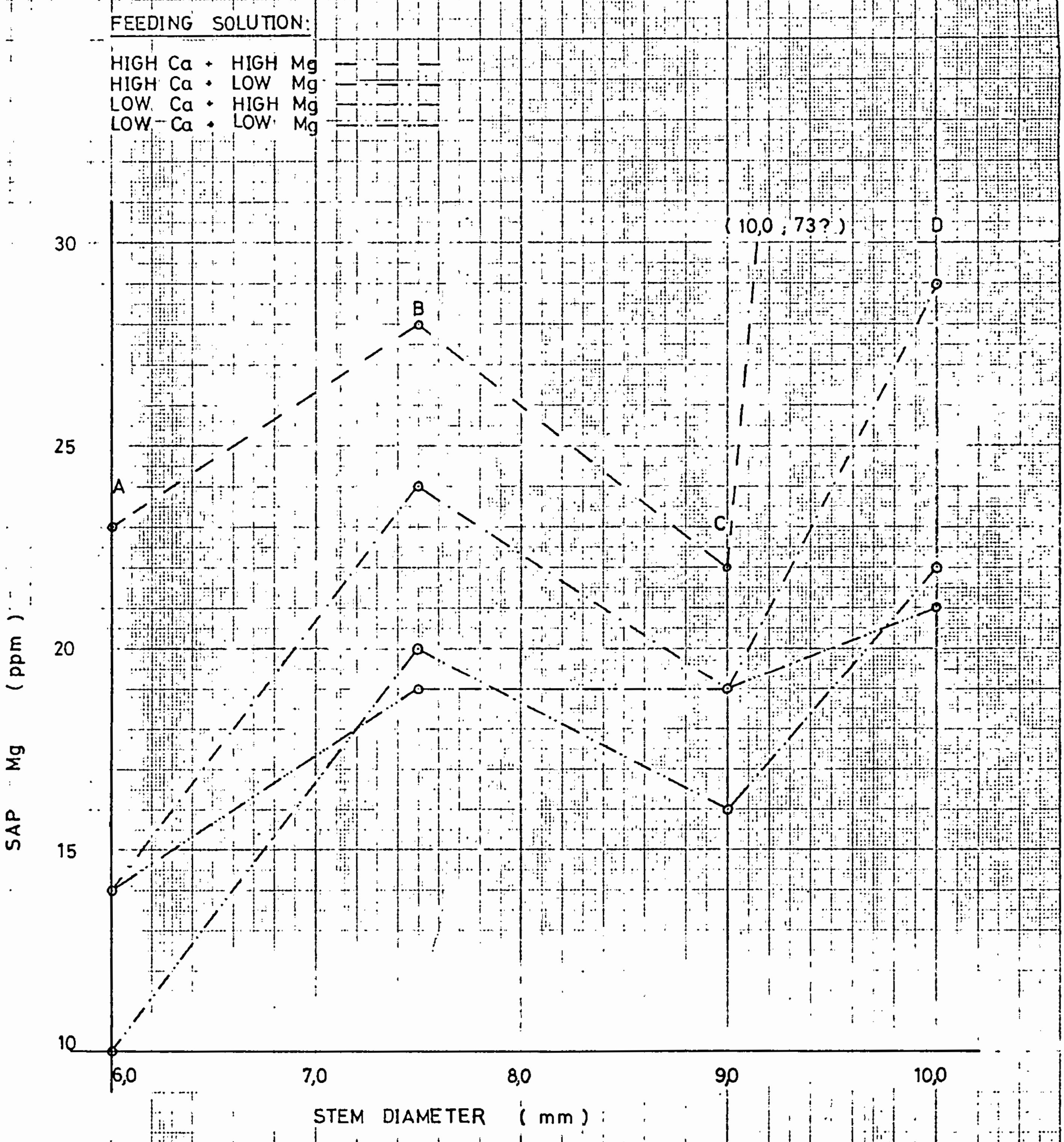
(c) FEEDING SOLUTION: LOW Ca + HIGH Mg



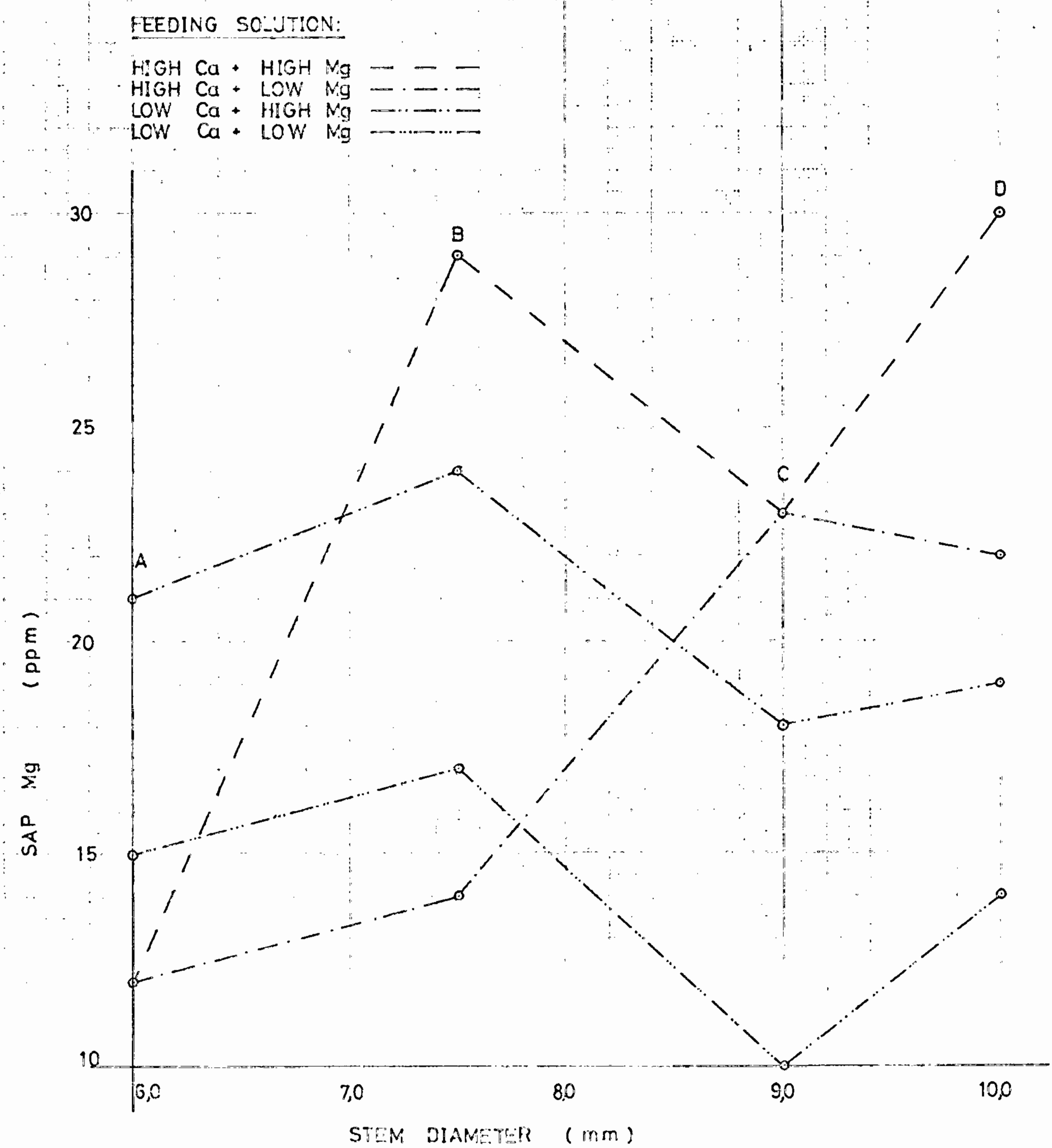
(d) FEEDING SOLUTION: LOW Ca + LOW Mg



GRAPH 3.16: SAP MAGNESIUM CONCENTRATION AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3(A).



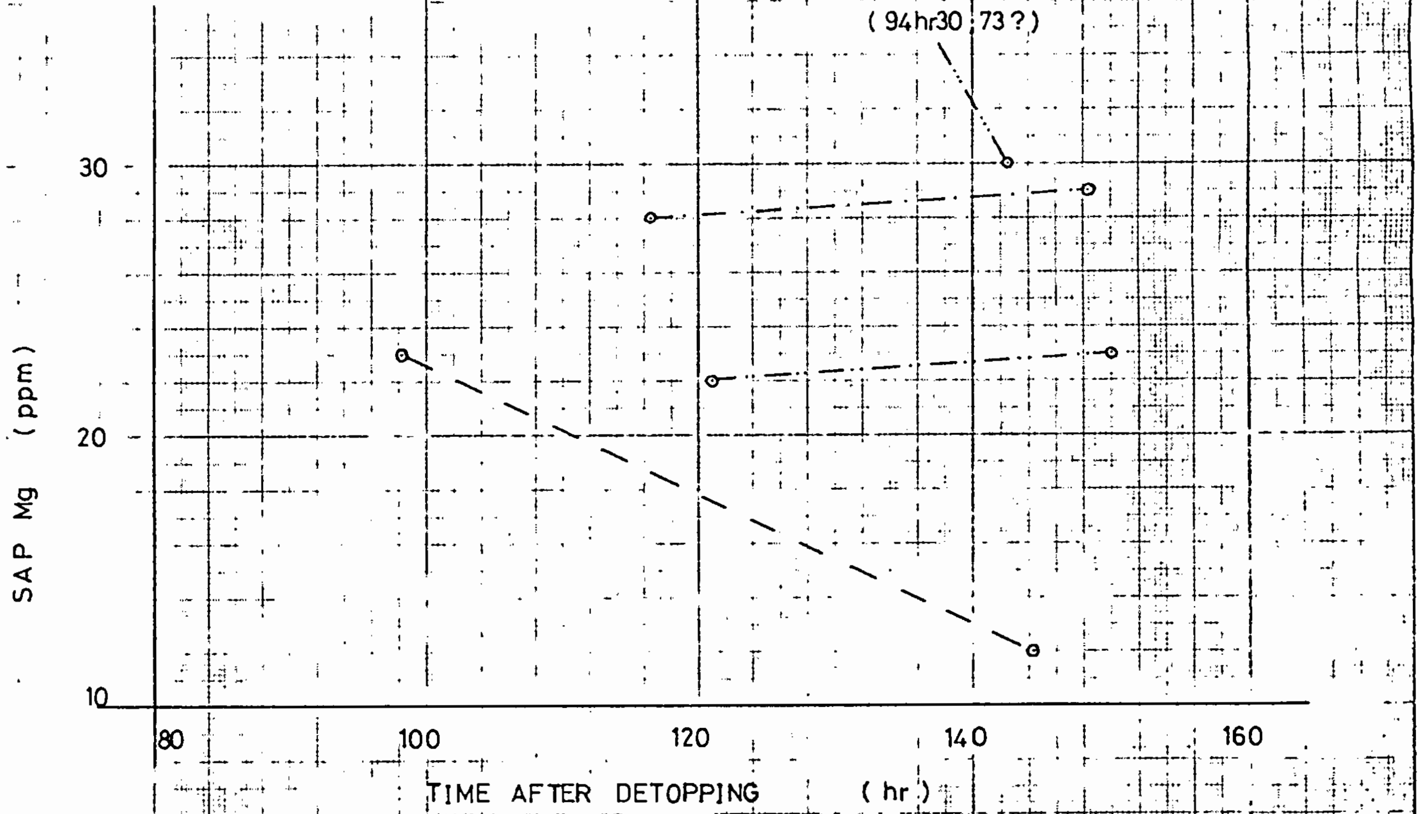
GRAPH 3.17: SAP MAGNESIUM CONCENTRATION AT DIFFERENT FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3(B).



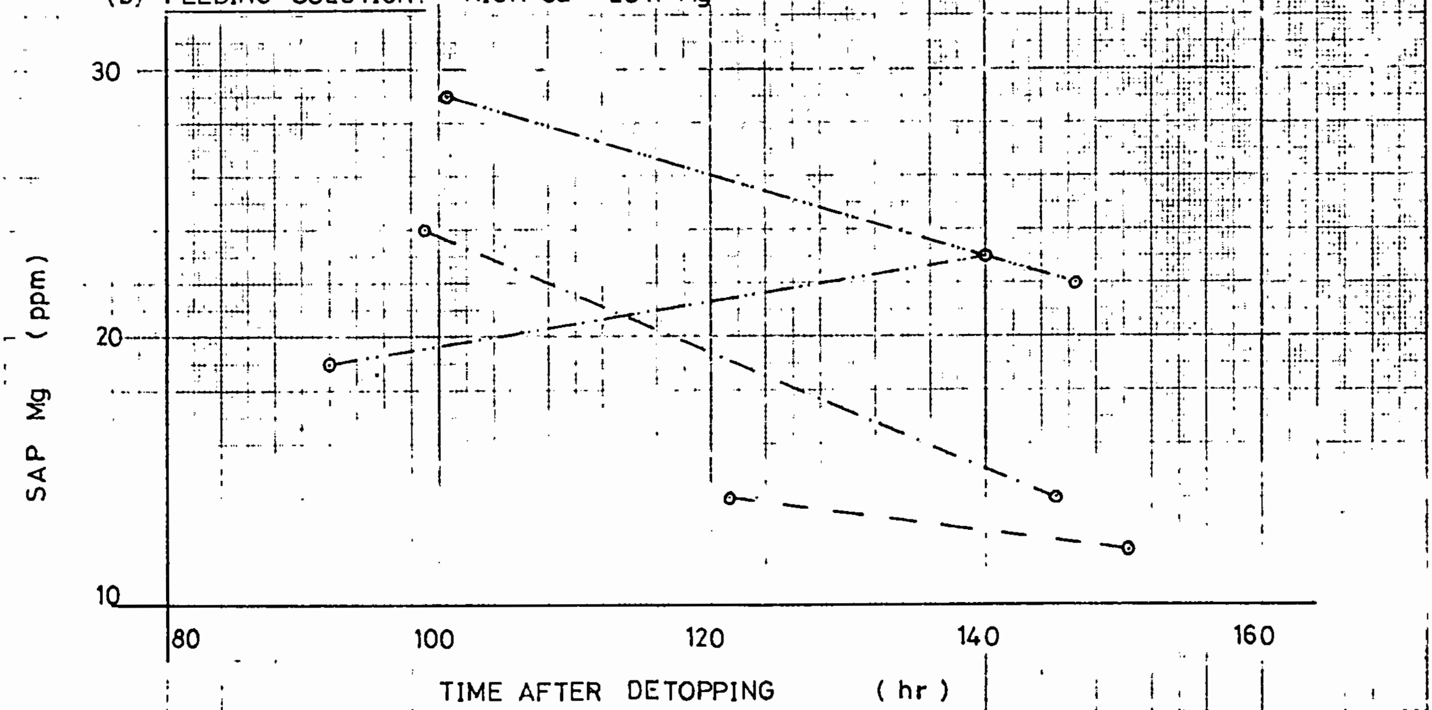
GRAPH 3.18(a): SAP MAGNESIUM CONCENTRATION AT SPECIFIC FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3.

PLANT. A — — ,B — · — ,C — ·· — ,D —···—.

(a) FEEDING SOLUTION: HIGH Ca + HIGH Mg



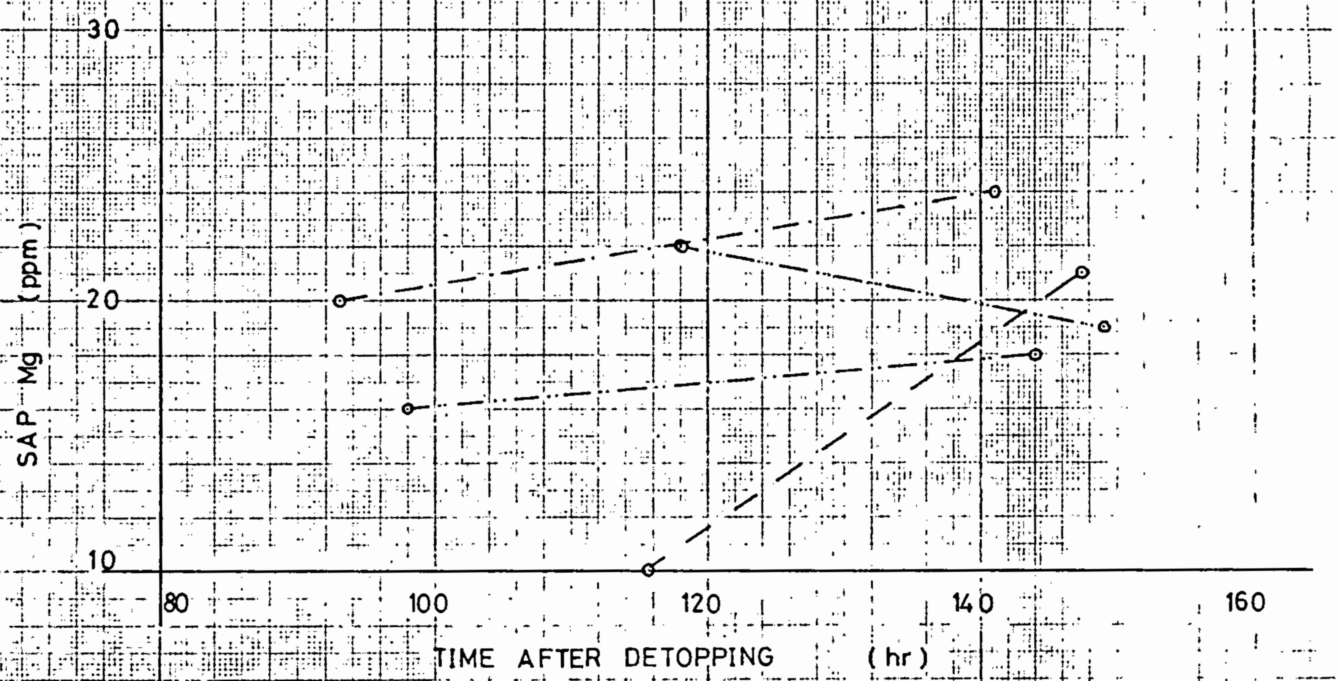
(b) FEEDING SOLUTION: HIGH Ca + LOW Mg



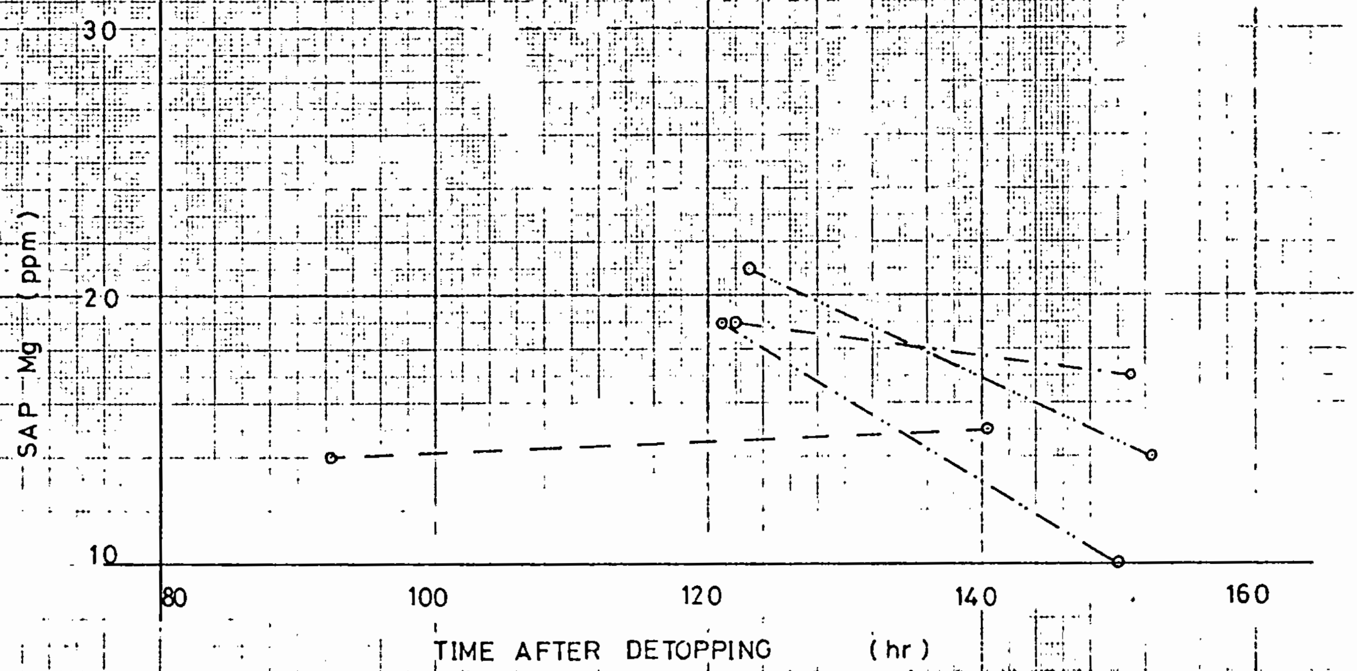
GRAPH 3.18(b): SAP MAGNESIUM CONCENTRATION AT SPECIFIC FEEDING SOLUTIONS AGAINST STEM DIAMETER FOR PLANTS A, B, C AND D IN EXPERIMENT 3.

PLANT: A — —, B —·—, C —··—, D ·····

(c) FEEDING SOLUTION: LOW Ca + HIGH Mg



(d) FEEDING SOLUTION: LOW Ca + LOW Mg



4. DISCUSSION OF THE RESULTS

4.1 Study of anatomical dimensions

Graph 3.1 and Table 3.1 clearly indicate that there is not a direct relationship between an increase in stem diameter and the xylem vessel area. The relationship appears to be exponential, (the vessel area increasing exponentially with respect to the stem diameter).

In order to account for the relationship between stem diameter and xylem vessel area in measurements of the rate of sap flow (as described by Table 3.2), sap flux was calculated. This would allow comparison of the effects of different feeding solutions on sap flux and sap calcium and magnesium concentrations as related to characteristics of root membranes and transport pathways in the different plants used. ?

4.2 The relationship between sap flux and the feeding solution

Consider first the effect of the four feeding solutions on sap flux in Experiments 1 and 2 (see Graphs 3.2 and 3.5 and Table 3.10). These experiments were both at -0,3 atm.

The sap flux values obtained for different size plants in Experiment 1 show a decreasing range from Plant B to Plant C. This means that a change in the calcium or magnesium concentration of the feeding solution had the most marked effect in Plant B (3,0 mm stem diameter). The ranking of feeding solutions as associated with high to low sap flux in Experiment 1 (see Table 3.10) show a greater similarity between Plants A and C than between A and B or C and B. In Plants A and C, (5,0 and 6,0 mm stem diameter respectively) the highest sap flux is associated with high Ca + high Mg and low Ca + low Mg. In Plant B however low Ca + high Mg give the highest sap flux. High Ca + high Mg and low Ca + low Mg give the lowest flux in this plant. Indeed the trend for high Ca + high Mg and low Ca and low Mg appears to describe an almost direct relationship between sap flux increase and stem diameter increase (for Plants B, A and C). Although the trend described by high Ca + low Mg and low Ca + high Mg is changed at Plant A (5,0 mm stem diameter), these conditions become more important for low flux as the stem diameter increases.

The sap flux values obtained for plants A, B and C in Experiment 2 are approximately ten times smaller than those of Experiment 1 (see Graph 3.5). The values obtained for the two water culture plants A and B are also less than half that obtained for the twelve day potted plant C. The range of flux values obtained for these plants is also much less than that in Experiment 1. Table 3.10 indicates that low Ca seems most important for high flux, while high Ca and high Mg give the lowest flux values.

In Experiment 3 (using 4 fresh plants at -1,0 atm vacuum pressure), Graphs 3.8, 3.9 and 3.10 show a marked similarity in sap flux trend for the different plants in the first and second runs (Experiments 3(A) and 3(B)). Results from Experiment 3(A) do however show a much greater range in sap flux values for individual plants. The values obtained in 3(A) are however generally more than twice as high as those in 3(B), indicating a substantial but surprisingly constant drop in the sap flux recorded for each plant between the two experiments (see Table 3.8 for time of feeding solution application).

Correlation of control run sap flux values with those obtained for the different feeding solutions in Experiment 3, show generally significant results (see Table 3.9 and Graph 3.7). In Experiment 3(A) HCa + LMg and LCa + HMg give the most significant correlation, while flux values for all feeding solutions gave significant correlations with the control sap flux. The meaning of the significant correlation is that within the flux range recorded for individual plants, there is a remarkably constant response to the different feeding solutions. The most consistent response is noted with high Ca + low Mg and low Ca + high Mg. High Ca + high Mg and low Ca + low Mg give the most different responses (particularly in plants A and D). These responses are nevertheless also consistent.

Table 3.10 further illustrates the remarkably consistent feeding solution ranking for individual plants as associated with high to low flux values for Experiments 3(A) and 3(B)(vertically on the table). Horizontally there is however a marked difference in ranking for different plants. In both 3(A) and 3(B) low Ca + low Mg are associated with the highest flux values, while low Mg seems to be important for low flux. Plants B and C, although having markedly different sap flux ranges (Graph 3.10), have fairly similar feeding solution ranking on Table 3.10. Low Ca + high Mg is associated with high sap flux, while low flux correlates with low Ca + low Mg (except in Plant C (3(A)) where HCa + LMg gives the lowest flux). Results for Plant D are very consistent with high Ca + high Mg ranking with high sap flux and low Ca + low Mg with low flux. (Value for 3(A) HCa + HMg in this plant was queried, but the use of dye to find membrane leaks gave negative results).

A comparison of feeding solution ranked against high sap flux for Experiment 3 does show some trend from Plant A (0,6 cm stem diameter) to Plant D (1,0 cm stem diameter). The trend shows increasing importance firstly of Mg and secondly of Ca. Conversely particularly high calcium becomes decreasingly associated with low sap flux values.

A comparison, firstly of actual sap flux values obtained for the three experiments and secondly ranking of the flux values against the respective feeding solutions show some interesting results.

Comparison of the sap flux values measured for Experiments 1, 2 and 3 show that those of the first and last are remarkably similar (that is, there appears to be no difference in the range). This illustrates that the smaller plants, although potted for one week, were capable of giving the same sap flux range as the larger fresh plants. Although there are marked differences in the actual range of sap flux values recorded for individual plants in Experiments 1 and 3, no clear relationship appears to exist with the stem diameter.

Sap flux values for the twelve day water culture and twelve day potted plants in Experiment 2 were, as already indicated, far lower than those obtained in Experiments 1 and 3.

A comparison of sap flux ranked against feeding solution in Experiments 1 and 3, (see Table 3.10) show that high Mg is constantly associated with high flux. Low sap flux, on the other hand, is generally associated with both low Ca + low Mg, (but in particular with low Ca). There therefore appears to be no clear difference in feeding solution requirements for high or low sap flux in the stem diameter range considered.

4.3 The relationship between sap flux and vacuum pressure

The sap flux values obtained for Plant C (Expt. 2) at different vacuum pressures, clearly illustrate that there is a certain vacuum pressure range over which relatively little increase in flux occurs, (Table 3.5 and Graph 3.11). This range is roughly between -0,25 atm and -1,0 atm and interestingly enough includes the two vacuum pressures used in Experiments 1, 2 and 3, (-0,3 atm in 1 and 2, and -1,0 atm in 3). The trend

Do not show this.

in Graph 3.11, although to be viewed cautiously, (as it was only observed in one particular plant), could account for the apparent similarity in sap flux range recorded for Experiments 1 and 3.

4.4 The effect of cyanide on sap flux

The effect of cyanide on sap flux was studied in Experiment 3 (see Table 3.6 and Graph 3.12). The general trend from 0 to 10^{-4} M KCN is similar for all plants. This decrease probably corresponds to the increase in membrane electrical resistance (as explained by Anderson et al, 1974). In Plants B and D this resistance drops abruptly beyond 10^{-4} M and the sap flux again rises. The second drop recorded in Plant B (between 10^{-3} and 10^{-4} M) does not fit the general pattern of flux increase and could be due to experimental error. A decrease in membrane resistance in Plant C only occurs after 10^{-3} M, and in Plant A it was not reached over the cyanide concentration range applied.

In all plants membrane characteristics regarding sap flux were markedly affected by the cyanide application. Subsequent measurement with ½ strength Hoagland feeding solution gave much higher and more irregular values than previously recorded and membranes seem to be damaged.

4.5 The relationship between sap calcium and magnesium concentration and that of the feeding solution

The sap Ca values obtained for different feeding solutions in Experiment 1 show a much smaller range in Plant B than Plants A and C (Graph 3.3). The ranking of feeding solutions as associated with high to low Ca concentrations are noticeably similar in Plants A and C, where high sap Ca is associated with high Ca (together with high or low Mg) feeding solution and low sap Ca with low Ca + low Mg. The relationship in Plant B seems rather anomalous as low Ca + high Mg are associated with high sap Ca and high Ca feeding conditions with low sap Ca. This tends to indicate that considerable accumulation may be occurring here (see Table 3.7). The difference between feeding and sap Ca is 48 ppm and 55 ppm for low Ca + high Mg and low Ca + low Mg respectively.

The Graph 3.4 of sap Mg obtained for each plant in Experiment 1 under the different feeding solutions, shows a fairly consistent response. The Mg range recorded in the sap again increases from B to C (as did the Ca range). The sap Mg values show a more consistent trend, with Table 3.10 indicating that high Mg in the feeding solution ranking with high Mg in the sap in all plants. In plant B, low Ca + high Mg was however responsible for a higher sap Mg reading than the high Ca + high Mg in plants A and C respectively. Low Ca + low Mg were associated with all low Mg measurements in the sap.

Comparison of feeding requirements for high and low Ca and Mg measurements in the sap shows that high Ca + high Mg is more important for high Ca and Mg in plants A and C than B. Low Ca + low Mg is associated with low Ca and Mg in all plants, except the anomalous behaviour in plant B where high Ca + high Mg gave the lowest sap Ca value.

In Experiment 2 (see Graph 3.6) an increasing stem diameter shows a fairly consistent increase in sap Ca concentration for individual feeding solutions. There is also a clear separation of the lines described by low Ca feeding solutions from those containing high Ca. A marked degree of accumulation appears to occur at low Ca feeding concentrations in Plant C (see Table 3.7). The difference between feeding and sap Ca is 48 ppm and 62 ppm for low Ca + high Mg and low Ca + low Mg respectively.

Table 3.10 simplifies the relationship between sap Ca and the feeding solution on Graph 3.7. High Ca as well as high or low Mg appears to be associated with high sap Ca, while low sap Ca is exclusively related to low Ca + low Mg feeding solutions.

Because of the very low volume of sap collected from plants in Experiment 2 (as is indicated by the low flux values), measurements of sap Mg could only be made for Plant C. In this plant high Ca + high Mg was associated with high sap Mg and low sap Mg, associated with low Ca + high Mg. This does not correspond to the feeding conditions related to low Mg in Experiment 1.

A comparison of sap Ca concentration measured for the different feeding solutions in Experiment 3(A) and (B) show markedly different trends for the two runs. In Experiment 3(A) there appears to be some separation of lines described by high Ca feeding solutions from those with low Ca. In fact there seems to be a general trend of increasingly high sap Ca being measured for the high Ca feeding solutions, while the lower Ca feeding solutions show a more horizontal trend.

In Experiment 3(B) all feeding conditions are however associated with a decrease in sap Ca with increasing stem diameter. Interestingly Plant A seems to have increased its sap Ca range (as indicated for increased sap Ca associated for high Ca + high Mg in Graph 3.15(a), and low Ca + high Mg and low Ca + high Mg in Graph 3.15(b)). All other plants show a marked decrease in sap Ca concentration for all feeding conditions with increasing experimental time, (except Plant C - for low Ca + low Mg, see Graph 3.15(b)).

A comparison of feeding solutions ranked for high to low Ca in Experiments 3(A) and (B)(Table 3.10) indicates that high Ca + high Mg is almost exclusively associated with high sap Ca, while low Ca + low Mg is related to low sap Ca.

The Mg concentrations measured for sap, as related to the different feeding solutions applied to the plants in Experiments 3(A) and (B), show a reasonably similar trend for high Ca + high Mg and high Ca + low Mg in the two runs (see Graphs 3.16 and 3.17). The trends for the other two feeding conditions are less similar.

Interestingly plants A, B and C (3(A)) have remarkably similar trends for high Ca + high Mg, high Ca + low Mg and low Ca + high Mg. The difference in these lines is remarkably constant between B and C (approximately 4 ppm). In Experiment 3(B) the line described by low Ca + low Mg also follows the high Ca + high Mg and low Ca + high Mg trend.

Graphs 3.18(a) and 3.18(b) show that the decrease in sap Mg measured during experimental time for different plants under the different feeding solutions do not show the same dramatic increases in sap Mg as recorded for sap Ca. Plant A does however show an increase in sap Mg with experimental time for low Ca + high Mg. The general trend appears to be one of constant sap Mg with increasing experimental time as was noted for Plants B and C with high Ca + high Mg and low Ca + high Mg (see Graphs 3.16, 3.17, 3.18(a) and 3.18(b)). Plant C showed a decrease in sap Mg (as with sap Ca) for all feeding solution conditions.

Ranking of feeding solutions for high to low sap Mg in Experiments 3(A) and 3(B) indicate that high Ca + high Mg is almost exclusively associated with high sap Mg (except for low Ca + high Mg in Plant A, Expt. 3(B)). Low Mg, on the other hand, is generally associated with low Ca, but not necessarily low Mg in the feeding solution.

A comparison of sap Ca values recorded for Experiments 1,2 and 3 show significantly higher concentrations in plants from the first two experiments (see Graphs 3.3 and 3.7). The high Ca concentrations here are, as would be expected, associated with high Ca feeding concentrations. Another interesting point is that the high Ca feeding conditions in Experiments 1 and 2 show the same trend as in Experiment 3(A), that is increasing sap Ca with increasing stem diameter.

Table 3.10 indicates that the high Ca feeding solutions generally rank with highest sap Ca in all plants from the three experiments. Low sap Ca is generally ranked with low Ca + low Mg feeding solution. No trend that may be related to an increasing plant size (stem diameter) is noted in Table 3.10.

Sap Mg values obtained for different feeding solutions in Experiments 1 and 3 show higher concentrations for Experiment 1 (similar to what was noted with Ca). In fact the range is from 17 ppm to 82 ppm in Experiment 1, as opposed to only 10 ppm to 29 ppm in Experiment 3 (see Table 3.7). High Ca + high Mg generally rank with the highest sap Mg measured in both Experiments 1 and 3 (Table 3.10). Low sap Mg is exclusively associated with low Ca + low Mg in Experiment 1, while the relationship appears less clear in Experiment 3 and can only be ranked with low Ca in the feeding solution. As with Ca, no trend in Mg ranking appears to exist that may be related to increasing stem diameter.

4.6 <u>The relationship between the sap flux and sap Ca and Mg concentrations as ranked against the root feeding solutions applied in each experiment</u> (see Table 3.10)

In the study of the relationship between sap flux and sap Ca and Mg concentrations as illustrated by ranking against feeding solution concentrations of Ca and Mg, the absolute values obtained have been effectively eliminated. Advantages in this ranking are that sap flux Ca and Mg may now be more directly related and thus reflect differences in the membrane characteristics of the range of plants used.

A comparison of feeding solution requirements for high sap flux and those for high sap Ca and Mg show that similar feeding solutions are associated with all three in Plants A, B and C in Experiment 1. The differences between these individual plants indicate that the smaller plant B (0,3 mm stem diameter) has a low Ca + high Mg requirement rather than the high Ca + high Mg requirement for high flux, Ca and Mg noted in the two larger plants. Judging from the vertical trend in the rank for Plant B this could well be related to Ca - Mg antagonism, with high Ca preventing high flux, Ca and Mg in this plant.

In Experiment 2 markedly different nutrient requirements appear to be associated with high or low sap flux, Ca and Mg. The high Mg feeding solution ranked against high flux in all three plants is associated with relatively low sap Ca and Mg. Interestingly, the high Ca ranked with low flux is associated with relatively high sap Ca and Mg (Mg measurement only in P_1ant C). The marked difference in feeding solution requirements for high sap flux and high sap Ca and Mg thus appear to separate root membrane characteristics associated with these plants from those of Experiment 1. Interestingly the potted plant C showed comparable feeding solution requirements for high flux and Ca to the water culture plants A and B.

Sap flux, Ca and Mg results from Experiment 3(A) and (B) show some trend with increasing stem diameter (horizontally across the table). High flux values are ranked against feeding solutions associated with relatively low sap Ca and Mg in the smallest Plant A (0,6 mm stem diameter). The relationship between high flux and high Ca + Mg seems to become somewhat stronger towards the largest plant D (1,0 mm stem diameter). According to this trend it appears that first high Mg" and then high Ca become important for high flux (these feeding conditions are however consistantly important for high sap Ca and high Mg). High Ca therefore again seems to prevent high flux (as with Plant B in Experiment 2), however this time it does not appear to affect high sap Ca or Mg to such a marked degree. (Low Ca + high Mg was only ranked against high sap Mg in Plant A in Run (b), where the vertical order of feeding solution indicates that there could well be some Ca antagonism of Mg uptake).

The horizontal relationship between low sap flux and low sap Ca and Mg on Table 3.10 appears less clear, although high Ca seems more important in the smaller Plant A than the larger plants where low Ca + low Mg are ranked most commonly.

Viewing Table 3.10 as a whole, the relationship between sap flux and sap Ca and Mg does not seem to indicate any trend with increasing plant size. Indeed the relationship between high sap flux and high sap Ca and Mg appears to separate the three experiments (as has been explained), while low flux and low Ca and Mg are more uniformly associated with the low Ca + low Mg feeding solution.

5. CONCLUSIONS

(1) The nutrient solution applied to the excised root system of Acacia cyclops at constant pressure has a significant influence on the sap flux measured in all plants.
(2) The range of sap flux values measured for one week potted and those for fresh plants of different sizes (stem diameter range from 0,3 to 1,0 cm), seemed to be remarkably similar and apparently not effected by the difference in vacuum pressure applied to the root systems. Adaptation to twelve days of water culture or potting was reflected by flux values which were approximately one tenth that obtained for the one week potted and fresh plants.
(3) Cyanide has a significant effect on membrane permeability, to water. This is reflected by a decrease and then increase in sap flux measured at a constant vacuum pressure.
(4) A comparison of feeding solutions ranked with high or low sap calcium or magnesium do not show any marked antagonism. High Ca + high Mg is generally associated with high sap Ca or Mg. This therefore indicates that high Ca in the feeding solution could enhance Mg uptake into the sap, as well as, high Mg enhancing Ca uptake.
(6) The relationship between sap flux and sap Ca and Mg concentrations for fresh plant root systems, as ranked with feeding solutions show the most interesting trend when correlated against increasing stem diameter. In smaller plants low Ca and low Mg feeding solutions are associated with high sap flux, as high Ca apparently inhibits it. High Mg and Ca feeding solutions become increasingly important for high sap flux in the larger plants. High Ca and Mg, and low Ca and Mg feeding solutions are however constantly associated with high and low Ca and Mg sap concentrations respectively.

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15 refs